October 30, 2009: Over 8 years ago I spoke to Jerome Bressler and thanked him for speaking out in this report. He told me the report was worse than what I had read because when the FDA had retyped it they left out the worst 20%, two mouse studies, and a cover letter. Doctors H. J. Roberts and Russell Blaylock both spoke to Bressler and got the same information.

Dr. Roberts wrote his Senator, Bill Nelson on November 27, 2001 stating that important information had been withheld. He said, "Specifically, I need original copies of the two (2) mouse studies done at Searle Laboratories, which were reviewed by the inspection team of the Chicago District of the Center for Food Safety & Applied Nutrition between April – September 1977.

Jane Kirby for Melinda Plaisier, Associate Commissioner for Legislation wrote Senator Bill Nelson on April 18, 2002 and said: "Additionally, some documents are considered confidential under FDA's FOI regulations and in some instances the Agency cannot acknowledge the existence of such documents."

The rest of the Bressler Report was kept under FDA seal for 3 decades. The investigation of these studies was the epitome of other Searle studies, sloppy, inefficient and never showed safety. The Bressler Report itself is revealing the things that Searle did so the FDA would not find out how unsafe aspartame is. They not only filtered out neoplasms but even excised brain tumors from rats, putting them back in the study and then resurrecting them on paper when they died. The report found that 98 of the 196 animals died during one of Searle's studies and weren't autopsied until later dates, in some cases over one year after they died. Records for approximately 30 animals showed substantial differences between original observations on pathology sheets and the observations on pathology sheets submitted to the FDA. There were numerous other inconsistencies. A uterine polyp and ovarian neoplasms were found in animals but not reported or diagnosed in Searle's reports. The FDA investigators found dose-related uterine polyps in 15% of 34 animals.

It was obvious even with fraud aspartame couldn't be proven safe and on January 10, 1977 in a 33 page letter, FDA Chief Counsel Richard Merrill recommended to U.S. Attorney Sam Skinner that a grand jury investigate Searle for "apparent violations of the Federal Food, Drug and Cosmetic Act, 21 USC 331 (e), and the False Reports to the Government Act, 18 U.S.C. 1001, for "their willful and knowing failure to make reports to the Food and Drug Administration required by the Act, 21 USC 355 (i) and for concealing material facts and making false statements in reports of animal studies conducted to establish the safety of (aspartame)."

U.S. Prosecutor Sam Skinner as well as William Conlon hired on with the defense team and the statute of limitations expired.

Finally in 1980 the FDA Board of Inquiry revoked the petition for approval which would have been signed into law if Searle had not sued. Donald Rumsfeld, CEO of Searle, hired to get aspartame approved, was on Reagan's transition team. FDA Commissioner Jere Goyan at 3:00 AM was called by a member of the transition team and fired. Reagan wrote an Executive Order making the FDA powerless to do anything about aspartame including signing the revoked petition into law until he could get Arthur Hull Hayes there as the new FDA Commissioner to over-rule the Board of Inquiry. Then the Executive Order was expunged from the record, which is illegal. This is mentioned in the movie, Sweet Misery: A Poisoned World, www.soundandfury.tv

So science never proved aspartame safe. It proved only fraud. But you hear the manufacturer constantly claiming there were 200 studies that proved safety. Informants say when Rumsfeld came to work for Searle people working there and knowing what was going on were fired and the studies were removed.

Jan Marie Kinnard, wrote in Feb, 2008 that she was the one who was hired to shred the Searle studies, and send a copy to France. She said: "They were the lab results from the tested rats and other animals. The results were outrageous. This stuff killed everything it touched."

The investigation of the two mouse studies the FDA did not want the public to read was kept from the record all these years. I even wrote FDA Freedom of Information and was told too it was confidential. When I stated it was not confidential but a matter of public record I was told that the information had been destroyed.

http://www.mpwhi.com/fda_gate.htm

Fortunately Dr. John Olney back in the 1970's had been able to get a copy of the deleted information and still had these records, which are now scanned in below to complete the Bressler Report. In one conversation with Jerome Bressler he said even Dr. Collins, chief FDA scientist's signature had been deleted from the report. He said the Bressler Report was not complete without it. An attempt to get Dr. Collins to speak on the subject was unsuccessful.

So below you will see the rest of the report and understand these mouse studies are just representative of the way Searle did studies, and there is no way for aspartame to ever be proven safe.

Dr. Betty Martini, D.Hum, Founder
Mission Possible International
9270 River Club Parkway
Duluth, Georgia 30097
770 242-2599

www.mpwhi.com, www.dorway.com and www.wnho.net
Aspartame Toxicity Center, www.holisticmed.com/aspartame

The missing 20% has been added to the end of the Report.

DU-A50

(9)

ESTABLISHMENT INSPECTION ENDORSEMENT

Page of Pages

1. ESTABLISHMENT

a. ESTABLISHMENT NAME
Searle Laboratories
Div. of G.D. Searle & Co.

b. DISTRICT
Chicago

c. CENTRAL FILE NO.

d. ESTABLISHMENT ADDRESS (Include Zip Code, Area Code and Telephone No.)
4901 Searle Parkway
Skokie, IL 60676

e. DATE INSPECTED
7/25/77-8/4/

2. ROUTING

a. HEADQUARTERS UNIT TO WHICH REFERRED (Use organizational symbol)
Bureau of Foods, HFF-330
Attn: Mr. Richard Ronk

HEADQUARTERS USE ON:
c. DATE REFERRED
PROFILE NOT NEEDED
BY [illegible] DATE 8-1

b. REASON FOR REFERRAL
To Be Reviewed by the Bureau of Foods

d. AF NUMBER

3. DISTRICT REMARKS

a. DISTRICT ENDORSEMENT

This top priority investigation was made to compare all available raw and summary data, along with all related material including methodologies, against the FDA submission. This inspection covers one study.

E-77/78 (P.T. 988573), SC-19192: 115 Week Oral Tumorigenicity Study in the Rat - Diketopiperazine

Study E-77/78 was initiated on November 8, 1971. The FDA submission is dated September 1974.

Three hundred and sixty weanling albino rats, Charles River CD strain, 180 of each sex, were used. The rats were divided into twelve housing groups, (six groups per sex), thirty rats in each housing group. Each housing group was composed of a random distribution of Control, Low, Mid, and High Dose animals. The rats were fed Diketopiperizine (SC-19192) at 0, 0.75, 1.5, and 3.0 grams per kilogram of body weight per day respectively

Our investigation of this study shows that no homogeneity tests were performed on any batches of the diet. We found evidence that the diets were not homogeneous.

Two unidentified infectious disease outbreaks were reported in the FDA submission. In both instances the control and treated animals were reportedly affected with equal frequency and severity. All morbid rats were injected with potassium penicillin G. Our review of the records show a third occurrence of infectious disease and penicillin administration took place, which was not reported in the submission to FDA.

We found an additional polyp of the uterus in the mid dose group which was not diagnosed or reported in the submission

b. REVIEWING OFFICER (Name and title)
Jerome Bressler

c. SIGNATURE
Jerome Bressler

d. DATE
8/7/77

e. DISTRIBUTION
O: CHI-DO CC: HFF-330, HFF-1, HFO-1, HFA-224, HFR-5140

FORM FD-Adv (11/75) PREVIOUS EDITION MAY BE USED UNTIL SUPPLY IS EXHAUSTED.

by Searle. The finding of one additional uterine polyp increases the incidence in the mid dose to 5 polyps of 34 animals (15%). The incidence of polyps of the uterus appears to be dose related.

Serum cholesterol determinations were done at days 796 and 798 (terminal bleeding), but not included in the submission to FDA. The submission reported a significant decrease in serum cholesterol that was more perceptible toward the end of the study and may have been dose related. Therefore, the exclusion of data from days 796 and 798 could be significant.

In some instances raw data was not available for review especially in the areas of clinical chemistry and microscopic pathology. In other instances there were inconsistencies in the raw data making it difficult to authenticate the study. As the investigation proceeded we learned that not all of the data was under seal. We discovered a number of documents. It is quite probable that there are still some laboratory notebooks missing. The majority of the responsible individuals that worked on this study are no longer with Searle.

FOLLOW-UP: To be reviewed by the Bureau of Foods

4/25/77 to 8/4/77

TABLE OF CONTENTS

Searle Laboratories
Div. G.D. Searle & Co.
4901 Searle Parkway
Skokie, Illinois 60076

SUMMARY OF FINDINGS

Authentication of this study was performed primarily by comparing available raw data with the submission to FDA. This was a problem, at times, due to the lack of some data and difficulty in locating other material. The majority of material relating to Aspartame was already under FDA seal at Searle. However, during this investigation we discovered various documents and notebooks that were not.

In some cases original data could be recorded in several areas, making it difficult, and sometimes impossible to determine which was actually the original. This was a particular problem in dealing with dates of deaths, as some conflicted on the "source" documents. Many of the responsible individuals involved with the study, including stability testing of DKP, are no longer employed by Searle. Dr. K.S. Rao, Study Monitor, the only individual who could have possibly answered some questions, had left Searle. He was contacted, but permission for an interview was refused by his attorney. Due to the absence of various individuals it was not always possible to accurately determine methods used in some analyses and operations carried out in conducting this study. In a number of areas, including chemistry, statistics, diet preparation and feeding, it was necessary to use assumptions, or information supplied by current employees who were not involved with the study.

At the beginning of this investigation, Mr. James R. Phelps, Vice-President and General Counsel for G.D. Searle & Co., advised us that an attorney and scientific coordinator would have to be present at all times to protect their interest in the data. This did not present any insurmountable problems, but on several occasions an attorney would question our request for data, stating that it was not relevant for authentication. At no time did we make any statement to the effect that our goal was to authenticate the study. Two memos were discovered dealing with reaction of animals to the diet. This was a significant factor in the study. Permission to copy them was initially refused, but finally granted after Searle was contacted by FDA General Counsel. We were not allowed to make xerox copies of any documents for about two and one-half weeks, due to Searle's concern over confidentiality. This was eventually reconciled between Searle and FDA General Counsel.

The major discrepancies concerning Study PT 988S73, SC-19192: 115 Week Oral Tumorigenicity Study in the Rat, are as follows:

A. Design & Conduct of Study

1) Control and treated animals were randomly distributed on the same rack. (See diagram of housing group attached as exhibit 7.)

2) No ear clips or other methods of uniquely identifying each animal were used. Identification consisted of two types of cards attached to the front of each cage.

3) Compound inventory cards were deficient in that only one of 18 such cards stated the purpose (study 988S73) for withdrawing the compound from inventory. Three of the cards did not include the date withdrawn, amount withdrawn, or signature of requestor. Therefore it was impossible to reconcile the amount withdrawn and the amount used. (See exhibit #28.)

4) Food jars were not individually identified, yet all the filled jars for a given housing group (control, low, mid, and high dose) were placed on a mobile cart, which was wheeled to the housing rack. The position of the jar (in rows) on the cart was the only means of identifying the proper dose level. The arrangement of the food cups on the cart is shown in exhibit #8.

5) A total of 79 "observations for drug effects" records were not signed or initialed.

6) Observation records indicated that animal A23LM was alive at week 88, dead from week 92 through week 104, alive at week 108, and dead at week 112.

7) Records indicated that at the scheduled 104 week bleeding, animal E2CM was substituted for A11CM. Records also indicated that animal A11CM was alive on this date and therefore should have been bled as scheduled.

8) Records indicated that penicillin was administered to four rats beginning on May 16, 1973, and continuing daily through May 28, 1973. This third occurrence of infectious disease and penicillin administration was not reported in the submission to FDA.

Searle Laboratories
Div. G.D. Searle & Co
Skokie, Illinois 60076

9) In many cases the actual number of tissues embedded was less than the 24 (control and high dose) or 19 (low and mid dose) specified in the final histology lab protocol dated 1/21/74.

10) Ophthalmoscopic examination records were present for animals H26MF and J29CM, yet the findings were not reported in the submission to FDA. Two other discrepancies of this type were noted.

11) Records indicate that a tissue mass measuring 1.5 X 1.0 cm was excised from animal B3HF on 2/12/72, and that a "skin incision over mass" was performed on animals C22LM and G25LM on Feb. 10, 1972.

B. Stability and Homogeneity of DKP in Diet Mixture

1) There were no batch records to show the quantities of DKP and basal diet weighed, type of mixer used, mixing time, dates, or names of individuals performing the weighing and blending operations.

2) There was no evidence that any tests had been done to determine the blending characteristics of the mixer, or to validate the mixing time.

3) No homogeneity tests were performed on any batches of diet used in the study, and two stability study assay reports (A7738) and A7739) indicated that samples were not homogeneous. (See exhibit #29.)

4) A stability study was conducted with DKP, in 1972. However, the 115 week rat study employed Basal Diet from week 62 to its conclusion, and no stability studies had been conducted with Basal Diet.

5) Methods of assay for DKP in the diet were deficient in that: The titration method was discontinued after 1 week of the stability study. Some of the TLC photographs showed no DKP reference standards and photographs also showed that there was something in the basal diet itself producing a spot on the TLC plate which had an Rf value corresponding to DKP. Only one solvent system was used for development of the TLC Plates. Some of the chromatograms showed poor separation.

6) No reserve samples of any of the lots of DKP used in this study were retained by Searle.

7) Three different sets of specifications for DKP were found, and Searle could not determine with any degree of certainty which of the three were applicable to the 7 lots of DKP used in the study.

8) The analytical records for DKP lots IR through 5R refer to reference standard IR #3701. None of the three sets of DKP specifications lists reference #3701. No data was made available as to dates, methods of preparation and authentication of DKP reference standards.

9) Analytical record A-9129 for DKP lot 5R showed an assay of 100.0%. Examination of laboratory notebooks showed that eleven (11) samples had been analyzed from this lot, and the analytical record only reflected an average of the last three of these. The other assays (not reported) ranged from 87.93% to 114.83%.

C. Dosage, Body Weight and Food Consumption

1) Examination of the raw data sheets revealed the following discrepancies:

   a. Empty feed cup weights were missing for the D housing group at the 12th week, in the raw data sheets. (See exhibit #75.)

   b. In several instances, the dietary concentration shown on the weight sheets did not agree with the concentration listed for the same level in the other housing groups. (For example; C group Males, mid & high levels for week 13,; A group Males, high levels for week 99)

2) Comparison of the Searle submission and the independent FDA analysis of the raw body weight and food consumption data revealed the following discrepancies:

   a. We found a total of 15 differences of 1 gram or more in the average body weight and of 0.1 percentage points or more in weight gain. (See table 1.)

b. We found approximately 82 discrepancies of one gram or more in the food intake when expressed in grams/day. (See table 2).

c. We found approximately 40 errors of 5 or more grams in food intake when expressed in grams/kg./day. (See table 2).

d. Most of our dosage calculations differed from Searle's dosage calculations by 10 or more mg., when the dosage is expressed as mg/kg/day. (See table 2).

D. Gross and Microscopic Pathology

1) 98 of the 196 animals that died during the study were fixed in toto and autopsied at some later date, in some cases more than one year later.

2) A total of 20 animals were excluded from the study due to excessive autolysis. Of these, 17 had been fixed in toto and autopsied at a later date.

3) Records indicated that animal F6HF, a high dose female, was found dead at 787 days of treatment and the gross pathology sheet reported a tissue mass measuring 5.0 X 4.5 X 2.5 cm. The submission to FDA reported no tissue mass and the animal was excluded from the study due to marked autolysis.

4) Records for approximately 30 animals showed substantial differences between gross observations on pathology sheets, when compared with the gross observations on pathology sheets submitted to FDA. A detailed description of 10 of these is included in the report. Copies of all the gross pathology sheets, and the pathology summaries submitted to FDA are attached as exhibits.

5) Dr. Charles H. Frith, D.V.M., Ph.D., Director, Pathology Services, NCTR, examined slides for a total of 150 animals, or about 42 percent of the animals on study. He noted the following discrepancies:

a. The reporting of a mass (by Searle) as missing which was actually present (animal M1LF).

b. The finding of a polyp of the uterus which was not diagnosed by Searle (animal K9MF). The finding of this additional uterine polyp by Dr. Frith increases the incidence in the mid dose to 5 of 34. (15 percent).

c. The finding of ovarian neoplasms in animals H10CF, H19CF, and H7HF, and the finding of diffuse hyperplasia in animal D29CF, which were not diagnosed by Searle.

d. The finding of additional inconsistencies in 21 animals.

6) No microscopic worksheets or other "raw data" relating to microscopic pathology could be found for this study.

7) A mammary tumor found in animal F27CF was described as a papillary cystadenoma on the pathology summary sheet, (page 105, Vol. II of the submission) and as an adenocarcinoma on summary table 12 (P. 96, Vol. I of the submission).

8) In several instances the histopathology technician made notes at the bottom of the gross pathology sheet to indicate that certain organs were not present in the bottle of fixative (and were therefore not available for sectioning). Yet, in three of these instances (animals A4CM , K23CF, and J3CM) a diagnosis appears in the submission to FDA.

E. Organ Weights

1) Organ weights were entered on the gross pathology sheets at the time of autopsy. We compared all of the individual organ weights on appendix table 5 in the submission to FDA (Vol. 1, pgs. 222-226) with the original data on the gross pathology sheets. A total of eleven (11) errors were noted in transcribing the raw data from the pathology sheets to the tables in the submission to FDA.

F. Survival

1. We were unable to determine the exact method used by Searle in constructing the survival table in the submission to FDA. We constructed a survival table using the body/feeder weight teletype sheets. A Life Table Analysis was constructed from our survival table by Dennis Wilson, FDA Department of Mathematics. The female control population differed from the high level population ($p < 0.05$) and the male control population differed from the mid and high level population (p 0.05). In all cases the differences are due to the higher mortality in the controls.

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Skokie, Illinois 60076

G. Clinical Laboratory Procedures

1. Laboratory records of one sort or another for all assays reported in the submission were obtained. In some cases data sheets were noted with results of assays carried out at treatment days not indicated in the submission Methods or Results section but indicated in the protocol or protocol amendment. For example, serum cholesterol determinations were done at days 796 and 798 (terminal bleeding) but not included in the submission to FDA. Because the submission to FDA (vol. 1 P. 286) reported a significant decrease in serum cholesterol that was more perceptible towards the end of the study, and may have been related to compound administration, the omitted data is of some importance.

2. No data was seen for two assays (serum insulin and serum ornithine carbamyl transferase) which were called for in an amendment to the protocol.

3. Original data was not always available for authentication of results or examination of procedures for conversion of raw data into the calculated values submitted to FDA.

4. Data pages for clinical chemistry and urinalysis were initialled by a technician who transcribed data but apparently was not directly involved in the assays indicated. He stated in an interview that Dr. Rao told him to intial the data sheets.

5. The methodolgy as referenced in the submission to FDA is incomplete and not always an accurate reflection of the methodology actually used in the study. The fact that more than one method was sometimes used for a particular assay during different times of the study was not indicated in the submission to FDA.

6. A total of 21 disparities between individual clinical laboratory analysis values appearing in the submission Volume I and those values appearing in data sheets and/or laboratory notebooks were found.

7. A total of 49 disparities were noted between statistical computations reported by Searle in the submission and those calculated by FDA. The disparities are constituted by the values for 6 means, 23 standard errors, and 20 significant differences (as measured by T tests).

8. Some of the data sheets for urinalysis had erroneously labeled the phenylketones test values as "phenylalanine".

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PURPOSE OF INVESTIGATION

Assignment memo dated May 16, 1977 from Donald Healton, Acting Director of Regional Operations, confirmed an earlier oral assignment to Chicago District for a directed inspection of certain non-clinical studies submitted to FDA in support of a food additive petition for the sweetner aspartame.

The investigation began on 4/25/77, and encompassed the authentication of all data, both raw and summary, relating to the studies jointly chosen for review by Bureau of Foods and EDRO. Two studies actually done at G.D. Searle were selected for initial coverage, and a decision to expand the investigation to a third study was made at a later date.

Following are the titles of the three studies selected for review:

1.) E-5 (P.T. #851S70), Evaluation of Embryotoxic and Teratogenic Potential in the Rat, conducted with SC-18862 (aspartame).

2.) E-89 (PT #1218S75), An Evaluation of the Embryotoxic and Teratogenic Potential in the Mouse, conducted with SC-18862 (aspartame).

3.) E-77/78 (PT #988S73), 115 Week Oral Tumorigenicity Study in the Rat, conducted with SC-19192 (diketopiperazine).

This report is concerned only with study E-77/78. The report of E-5 and E-89 was submitted separately.

HISTORY OF BUSINESS

G. D. Searle & Co. provides a wide range of health care products and services on a worldwide basis. Its business is divided among three principal areas: pharmaceuticals, medical instruments and optical products, and hospital and laboratory products. The firm's corporate offices are located in Skokie, Illinois, with various branches and facilities throughout the world.

Effective June 1, 1977, Donald H. Rumsfeld assumed duties as President and Chief Executive Officer. Mr. Daniel C. Searle, formerly Chief Executive Officer is now Chairman of the Board, while William L. Searle and Wesley M. Dixon, former Chairman and President respectively, are now Vice-Chairmen.

Effective March 1, 1977, the firm underwent a major realignment, shifting to a managerial system based on product lines. This resulted in the establishment of four main product-line groups, which are: Pharmaceutical/Consumer Products, Diagnostics, Hospital Supplies and Optical Products. Each group is headed by a President who will report to Searle's Executive Vice-President for Operations, Dr. James A. Buzard. A copy of the G. D. Searle & Co. annual report for 1976 which is attached as Exhibit #1 further expands on the firm's operations and lists Corporate Officers.

Mr. O. B. Parrish is President of the Pharmaceutical/Consumer Products Group and also a Corporate Vice-President. An organizational chart for this group is attached as Exhibit #2. Mr. Guy Labrosse is now Group Executive Vice-President for U. S. Commercial Pharmaceutical Operations. In the U. S., this is known as Searle Laboratories. The facility at 4901 Searle Parkway, Skokie, Illinois is a part of U. S. Operations, e.g. Searle Laboratories, yet houses the majority of the Research and Development Group.

Worldwide Pharmaceutical Research and Development is also a part of the Pharmaceutical/Consumer Products Group, but not of Searle Laboratories. The Research and Development of Aspartame is a function of this group. Copies of organizational charts for this group are attached as Exhibit #3. Dr. Robert A. Moe recently resigned and his position is temporarily being filled by George V. O'Bleness, Corporate Vice-President for Compliance and Administration.

Commercial aspects of Aspartame are being handled by an "Aspartame Division", under the direction of Elwood H. Ensor, Corporate Vice-President. There is no longer a division entitled "New Ventures".

PERSONS INTERVIEWED

Credentials were shown and a written Notice of Inspection was issued to Dr. William M. Merino, Director, Domestic Pharmaceutical Products, Regulatory Affairs Department on April 25, 1977. The following Searle personnel were present at the initial meeting on 4-25-77.

Robert A. Moe, PhD. - Executive Vice-President
George Clay, PhD. - Group Leader, CNS Pharmacology
Robert Bost, PhD. - Director of Food Products, Regulatory Affairs
Holly Ru Probst- Director, Corporation Information Management Group
Dave Britton - Director Corporation Information Department
William Merino, PhD. - Director, Domestic Pharmaceutical Products
Richard Viktora - Attorney
James Phelps - Vice-President, General Counsel
Elwood H. Ensor, PhD. - Vice-President
Paul Klimstra, PhD. - Vice-President Pre-Clinical Research and Development
Roger Thies - Attorney

During the course of our investigation one or more of the following Searle personnel were present in the Conference Room which we used for our data review.

Richard Viktora - Attorney
Roger Thies - Attorney
George Clay, PhD. - Group Leader, CNS Pharmacology
Robert Bost, PhD. - Director of Food Products, Regulatory Affairs
Don Cook, PhD. - Associate Director, Department of Bio Research
Dick Aspinol, PhD. - I. I. D. Group Leader
Bill Jenkins, PhD. - Director, Product Affairs
Fred McIlreath, PhD. - Director, Regulatory Affairs
Paul Landefeld, Attorney

Most of the time one attorney (R. Viktora or R. Thies) and one scientist were present. During our initial meeting with Searle personnel, James Phelps stated that a Searle monitor must be with us at all times during our data review in order to "protect the data".

During the course of our investigation, various individuals were interviewed in an attempt to obtain all available raw data and reconstruct the manner in which the study was conducted, as accurately as possible. Since many employees involved in the study or support areas are no longer employed at Searle, others were interviewed who had general knowledge of such parameters as statistics and chemistry.

Significant interviews are attached as Exhibits, as referenced. Individuals interviewed were as follows:

1. Donna Helms - Administrative Assistant to Dr. McConnell on 5-18-77, 6-30-77 and 7/1/77 (Exh. #46).
2. Judith Beauchamp - Hematology Lab Supervisor on 6-2-77 (Exh. #47).
3. Barbara Bickford (Nee Ross)-Technician, Department of Analytical Research on 6-1-77 and 6-2-77 (Exh. #48).
4. Clifford J. Seul - Supervisor, Department of Analytical Research and Development on 6-2-77 (Exh. #49).
5. Bartolome R. Tangonan - Research Technician, Pathology Toxicology Department on 6-1-77 (Exh. #50).
6. Tony Martinez - Research Assistant and Toxicology Lab Supervisor on 5-19-77, 6-3-77, 7-7-77, 7-20-77 and 8-2-77 (Exh. #51).
7. Ted Reichert - Supervisory Systems Analyst on 5-24-77 (Exh. #52).
8. Phil Polli - Systems Analyst on 5-24-77 (Exh. #53).
9. Judith Schmal - Clinical Chemistry Section Supervisor on 6-2-77 and 6-7-77 (Exh. #54).
10. Jane Drury -Analytical Chemist, Bioanalytical Dept. 6-7-77.
11. Alan Mitchell - Teratologist on 7-20-77 (Exh. #56).
12. Raymond G. Schroeder - Former Searle Teratologist on 7-18-77 (Exh. #57).
13. Dr. Rudolph Stejskal - Pathologist on 6-23-77.
14. Patricia Erdenberger - Research Assistant and Histopathology Lab Supervisor on various dates (Exh. #58).

Dr. Robert McConnell, Pathology-Toxicology Advisor at the time of this study, was not directly involved with daily procedures. He is no longer employed at Searle.

An attempt was made to interview Dr. K. S. Rao, Monitor of Study P. T. #988S73 on 7-25-77. We were referred to Dr. Rao's attorney, who refused permission for an interview (see Jerome Bressler's memo dated 7-27-77, Exh. #33).

PURPOSE OF STUDY PT 988S73 (E-77/78)

SC-19192: 115 Week Oral Tumorigenicity Study in the Rat

According to the submission to FDA, this study was intended to evaluate the safety and tumorigenic potential of SC-19192, diketopiperazine (5-benzyl-3, 6-dioxo-2-piperazine-acetic acid), which is a conversion product of aspartame, and to induce and define such adverse effects as might occur only at prodigious multiples of the estimated daily human intake. The commercial grade of aspartame (SC-18852) may contain up to 2 percent of the conversion product (DKP), according to Searle's specifications.

DATES

Study E-77/78 (PT #988S73) was initiated on November 8, 1971. The study was to be terminated at 104 weeks, but was extended to 115 weeks. The reason for extending the study was stated as follows in protocol amendment #3 dated September 6, 1973: "it was decided to extend or continue the study until the mortality of either sex reduced the control group to 20 animals per sex, provided the survival in the treated groups is not less than 10 animals/sex/treated group prior to that period. This approach is consistent with current FDA desires." A copy of the study protocol is attached as exhibit #11.

Initiation of treatment was staggered over a two week period as follows:

HOUSING GROUP	DATE PLACED ON STUDY	SCHEDULED SACRIFICE	DAYS ON STUDY
A - Male	11/8/71	1/21/74	805
B - Female	11/9/71	1/22/74	805
C - Male	11/9/71	1/22/74	805
D - Female	11/10/71	1/23/74	805
E - Male	11/11/71	1/24/74	805
F - Female	11/12/71	1/25/74	805
G - Male	11/15/71	1/28/74	805
H - Female	11/16/71	1/29/74	805
J - Male	11/17/71	1/30/74	805
K - Female	11/17/71	1/30/74	805
L - Male	11/18/71	1/31/74	805
M - Female	11/19/71	2/1/74	805

PROTOCOL AND AMENDMENTS

A copy of the protocol for this study was obtained and is attached to this report (See Exhibit #11). The protocol includes 4 amendments which are dated Aug. 20, 1973, (amendments #1 and 2), Sept. 6, 1973 and Jan. 9, 1974.

Amendment #1 dated Aug. 20, 1973 specified 4 additional clinical chemistry laboratory measurements: 1.) serum insulin, 2.) serum ornithine carbamyl transferase, 3.) serum protein electrophoresis, 4.) serum total protein.

Two of the above assays (serum insulin, and serum ornithine carbamyl transferase) were apparently not done, because no data for these two parameters was submitted to FDA, and we could find no raw data or other evidence that they were done.

Amendment #2 dated Aug. 20, 1973, specified 8 coronal sections of brain to be examined microscopically, and also described the procedure for sectioning the urinary bladder. Four transverse sections from each urinary bladder were to be examined microscopically.

Amendment #3 dated Sept. 6, 1973 extended the study until it reached a point where mortality reduced the control group to 20 animals per sex, provided survival of treated groups was not less than 10 per sex per group. (This represented a survival of approximately 30%).

Amendment #4 dated Jan. 9, 1974 added serum cholesterol to the clinical chemistry measurements to be made at terminal sacrifice, and terminated the study after 114 weeks of treatment. Terminal sacrifice was to begin on 1-24-74 and continue through 2-1-74.

Our examination of the original data showed that serum cholesterol determinations were done at day 796 and 798 (terminal bleeding) as specified in the above amendment, but the data was not included in the submission to FDA. The submission to FDA (Vol. 1 p. 286) reported a significant decrease in serum cholesterol that was more perceptible towards the end of the study, and may have been related to compound administration. Therefore, the omitted data may have been important.

Serum cholesterol determinations were also done at day 546 (78 weeks) and not reported in the submission to FDA.

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The protocol for Clinical Chemistry procedures specified that BUN determinations were to be done at 78 weeks (546 days). The submission to FDA contained no BUN data for day 546, but our review of the raw data indicated that BUN's had been done at day 546. Some BUN's were also done at day 735 (105 weeks) and not reported in the submission to FDA, but this data was not complete for all animals.

Attached to the protocol is a memo dated Oct. 31, 1972 which describes an acute infection spreading in the rat colony, and the administration of penicillin to combat the infection, and a memo dated May 8, 1973 listing scheduled dates to be added to Body and Feeder Weights of housing groups A & B.

The final Histology Lab Protocol, dated 1-21-74, specifies 24 organs to be embedded for control and high dose animals, and 19 organs to be embedded for low and mid dose groups. The organs which were to be embedded for the control and high dose groups but to be omitted in the low and mid dose groups include: lymph node, nerve, bone, eye, and salivary glands.

Pathology sheets (blank forms) to be used at terminal sacrifice were reproduced (xeroxed) with check marks, time (death to tissue fix), fixative, study, and project number already entered. Twenty-seven (27) organs were checked off, to be embedded. However, as stated above, the control and high dose animals were to have 24 organs embedded, according to the protocol, and the mid and low dose 19. Therefore, all pathology sheets for animals killed by design have incorrectly identified the specific organs and tissues to be embedded.

In addition to the above error, in many cases the actual number of tissues embedded was less than the 24 (control and high dose) or 19 (low and mid dose) specified in the final Histology Lab Protocol dated 1-21-74. Specific figures for numbers of tissues embedded at terminal sacrifice are as follows:

	ACTUAL RANGE	ACTUAL AVERAGE	NUMBER SPECIFIED IN PROTOCOL	NO. OF ANIMALS NOT IN ACCORD WITH PROTOCOL
CONTROLS	10-24	20	24	129 of 144
LOW DOSE	12-23	19	19	19 of 72
MID DOSE	4-24	18	19	28 of 72
HIGH DOSE	9-25	22	24	51 of 72

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PERSONNEL AND RESPONSIBILITY

The names of Dr. K.S. Rao, Dr. R. Stejskal, and Dr. R.G. McConnell appear on the final study report, indicating that they are the authors of the report, and were responsible for the study.

Following are the principal persons involved with study E-77/78 and their specific areas of responsibility:

1.) Dr. Robert G. McConnell - Director, Pathology-Toxicology Section 1970 through 1974. Dr. McConnell functioned as the Path-Tox advisor on study E-77/78. He is no longer employed by Searle.

2.) Dr. Suryanarayana K. Rao - Manager, General Toxicology Laboratory, June 1971 until he left Searle in May of 1977. Dr. Rao was the Path-Tox monitor for Study E-77/78. In 1971 Dr. Rao monitored 30 studies, in 1972 forty-seven (47) studies, in 1973 twenty-nine (29) studies and in 1974 twenty-five (25) studies.

3.) Dr. Rudolph Stejskal - Senior Research Investigator, Pathologist. Dr. Stejskal was responsible for the microscopic findings and accuracy of these findings in the study report of E-77/78. Because Dr. Stejskal joined Searle in July, 1973, he had no input into the pathology protocol. Also, he did not examine all of the slides for this study, but was assisted in that task by Dr. Joseph H. Smith M.D.

4.) Dr. Joseph H. Smith, M.D. - Group Leader and Senior Pathologist at Michael Reese Hospital, Chicago, IL., before joining Searle in June of 1973. Dr. Smith examined some of the slides for Study E-77/78, and supervised the necropsy laboratory.

5.) Tony Martinez - Toxicology Laboratory Supervisor, 1970 through 1973. Mr. Martinez participated in twelve (12) studies in 1971, Seventeen (17) studies in 1972, and thirteen (13) studies in 1973. Mr. Martinez supervised the technicians who worked on study E-77/78 and was responsible for the day-to-day conduct of the study. He also performed some necropsies.

6.) David K.T. Kie, B.S., Research Assistant in Pathology Laboratory. He performed some of the necropsies on E-77/78.

7.) Robert Spaet - Research Assistant. He also performed necropsies.

8.) Bartolome R. Tangonan - Research Technician II - He was involved with preparation of diet mixtures, daily observations, weighing and feeding animals, etc.

9.) Donna K. Helms - Manager, Safety Evaluation, Project Scheduling, Reporting, and Data Storage, Path-Tox Dept. and Administrative Assistant to Dr. McConnell.

10.) Patricia Erdenberger - Research Assistant, and Histology Lab Supervisor.

11.) Dr. Eugene Joseph Youkilis - Senior Research Investigator. He performed the ophthalmoscopic examinations in study E-77/78.

12.) Judy A. Henderson - August 1972 to present, Research Technician III, Histopathology Dept. She was involved with tissue processing on study E-77/78.

13.) Judith R. Schmal - Nov. 1971 to present, Supervisor, Clinical Chemistry Section of Bioanalytical Laboratory.

14.) Judith A. Beauchamp - Employed Aug, 1970 to present; Supervisor Hematology Laboratory since April 1973.

15.) Barbara (Ross) Bickford - Research Technician, Quality Control Department. She performed analyses of DKP diet mixtures for study E-77/78.

16.) Clifford J. Seul - Supervisor, Method Development, Stability Evaluation Laboratory. He was Barbara Bickford's supervisor at the time the DKP stability study was performed.

17.) Jack Drogt - 1967 to present, Senior Research Assistant, Chemical Development. Mr. Drogt manufactured the 7 lots of DKP used in study E-77/78.

18.) Dr. John E. Dutt - Math-Stat. Dept.

19.) John Mellman, Math-Stat Dept.

20.) Fred Hunter, Technician.

Since the Task Force investigation in 1975, there has been a major internal reorganization. The current organization of Worldwide Pharmaceutical Research & Development is attached as Exhibit #3). The only change has been the resignation of Dr. Robert A. Moe, Executive Vice-President. Mr. George V. O'Bleness, Corporate Vice-President, is temporarily filling this position.

Organizational charts for Preclinical Research and Development of Product Safety Assessment are also attached as Exhibits 4 & 5. There have been no changes in these areas to date.

Worldwide Pharmaceutical Research and Development is responsible for research and development of Aspartame and is a part of the Pharmaceutical/Consumer Product group. The group President is O.B. Parrish, who reports to James A. Buzard, Executive Vice - President for Operations, G.D. Searle & Co. The current corporate structure of G.D. Searle & Co. has been discussed under History of Business.

P.T. No. 988S73, 115 Week Oral Tumorigenicity Study in the Rat was conducted between November 1971 and February 1974. The final FDA submission was dated September 1974. Following is a yearly breakdown of key personnel during this study:

1971

Robert Moe - Director, Biological Research Department.
Robert McConnell - Director, Pathology - Toxicology Section
K. S. Rao (June, 1971) - Manager, Toxicology Laboratory.
Tony Martinez - Toxicology Laboratory Supervisor.

1972

Robert Moe - Director Biological Research Department (January through April).

F. Saunders - Director, Biological Research Department (May through December).

Robert McConnell - Director, Pathology-Toxicology Section

K.S. Rao - Manager, Toxicology Section.

Tony Martinez - Toxicology Laboratory Supervisor.

1973 (January to June)

Francis Saunders - Director, Biological Research Department.

Robert McConnell - Director, Pathology-Toxicology Section.

K.S. Rao - Manager, General Toxicology Laboratory.

Tony Martinez - Toxicology Laboratory Supervisor.

1973 (July to December)

Paul Klimstra - Director, Pre-clinical Research & Development Department.

Robert McConnell - Director, Pathology-Toxicology Department.

K.S. Rao - Manager, General Toxicology Department.

Tony Martinez - Manager, General Toxicology Laboratory.

1974

Paul Klimstra - Vice President, Pre-clinical Research & Development.

Robert McConnell - Director, Pathology-Toxicology Department.

K.S. Rao - Manager, General Toxicology Laboratory.

D.Semler - Toxicology Laboratory Supervisor.

A more complete listing of personnel in the Department of Science, from 1971-1975 is attached as Exhibit No. 64. This includes the Pathology - Toxicology Department and other ancillary areas.

Curriculum vitae for individuals performing significant functions in the study are attached as Exhibit 12.

MANUFACTURE AND TESTING OF SC-19192

Seven batches of SC-19192 (diketopiperazine) were used in this study. All batches were manufactured in-house by Searle Chemist Jack Drogt. The lot numbers, analytical numbers, and quantities are as follows:

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Lot Number	Analytical Number	Quantity (After Milling)
1R	6906	
2R	7274	
3R	7273	
4R	7291	
5R (JDR-5-18A)	9129	
6R (JDR-5-30A)	9805	
7R (JDR-5-30B)	9829	
	Total	

Batch records covering the manufacture of lots 1R through 5R were reviewed. Batch records for lot 6R and 7R could not be located by Searle personnel. Analytical reports for all seven batches were reviewed. Copies of the batch records and analytical records were obtained and are attached to this report, along with copies of pages from Jack Drogt's laboratory notebook, and other laboratory notebooks relating to the analysis of lots 1R through 7R of DKP. (See Exhibits 13-23.)

We obtained copies of three different specification sheets for DKP. (See exhibits 16-18.) We could not determine with certainty which of the three specification sheets was in effect at the time that the 7 lots of DKP used in this study were assayed, because only one of the three specification sheets was dated. This resulted in ambiguities for two of the parameters measured: melting point and identity (IR Spectrum). Specification memorandum dated Dec. 4, 1969 listed a melting range of 252-256 degrees C. Another specification sheet (not dated) entitled "Tentative Specification For SC-19192" listed a melting range of 241-246 degrees C. A third specification sheet entitled "Specifications for SC-19192, Specification No. C 40606C" (not dated) listed a melting range "at about 243 degrees C."

For identity (IR Spectrum) the first sheet (dated 12/4/69) specified that "The reference standard shall be considered to be TJT-12-32 until something better comes along". The second and third sheets specify that the DKP "Conforms to IR #2358".

No data was made available as to dates, method of preparation and authentication of DKP reference standards used.

Searle attorney Roger Thies was contacted about this point Aug. 1, 1977 and said he would attempt to obtain information regarding this point but later registered doubt as to whether anything would be found.

We asked Searle personnel to tell us which of the specification sheets was valid for the DKP used in study E-77/78. We were

told that the third sheet, identified with "No. C4060C", could not have been used since the number corresponded to a date in June, 1974.

It is not clear as to the exact date that the first sheet (dated 12/4/69) was superceded by the second one, identified "tentative specifications for SC-19192" because the second sheet was not dated or numbered. However, Searle Attorney Roger Thies told us that their "best guess" was that the sheet marked "tentative specifications for SC-19192" was the one used.

Accordingly, we have used the specifications from the sheet marked "tentative specifications" for the following chart, which compares the specifications with the actual results of analysis.

	DXP LOTS						
SPECIFICATIONS	1R	2R	3R	4R	5R	6R	7R

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DKP LOTS (Continued)

SPECIFICATIONS	IR	2R	3R	4R	5R	6R	7R

The only discrepancy apparent in the above chart is in the criteria for identity. The specification lists reference standard IR #2358, while the analytical record for lots through 5R refer to Refererence #3701.

Examination of the laboratory notebooks referenced on the analytical records revealed other possible discrepancies. For example, the analytical record A-9129 for DKP Lot 5R showed an assay (titration) of 100.0 percent. The analytical record referenced two different lab notebooks assigned to two different analysts. Examination

of lab notebook AR-68 assigned to Sandra Ann Carey revealed that she had analyzed 3 samples of lot 5R on 11/9/72. Results of the analysis showed that sample one had an assay (by titration) of 89.70 percent, sample two, 87.93 percent and sample three was discarded.

Apparently not satisfied with her results she repeated the assay on the same day (11/9/72) and obtained 93.23 percent (the average of 3 samples), still well below the specification of 99.0 percent. The other lab notebook referenced was AR-57, assigned to E. Aranda. This notebook showed that analyst Aranda performed an assay (titration) of lot 5R on 12/1/72 the results of which were 114.83 percent for 3 samples. Apparently not satisfied with the results, he repeated the assay on 12/6/72 and obtained 100.4, 99.9, and 99.8 percent for an average of 100.0 percent. This result (100.0 percent) was the only one reported on the analytical record A-9429.

The analytical record (A-7291) for DKP lot 4R shows a result of "less than 20 PPM" for the heavy metals test. Two laboratory notebooks are referenced: VSH-I, pages 260-263, and AR-23, page 269. Examination of both of these books revealed no mention of a heavy metals test.

The analytical record (A-9805) for DKP lot 6R (JDR-5-30A) also showed a result of "less than 20 PPM" for the heavy metals test. Examination of the referenced laboratory notebook (AR-77, page 83-86) revealed no evidence of a test for heavy metals.

The analytical record (A-9829) for DKP lot 7R (JDR-5-30B) again showed "less than 20 PPM" heavy metals. Examination of the referenced lab notebook (AR-93) again showed no evidence of a heavy metals test.

The above discrepancies were the only ones noted with respect to lots 1R through 7R of DKP. All other criteria for identity and purity of DKP as shown in the reports of analysis, conforms to Searle specification sheet marked "tentative specifications for SC-19192". It should be noted however that none of the seven lots of DKP met the specifications on sheet dated 12/4/69, with respect to melting range.

STABILITY AND HOMOGENEITY OF DIET MIXTURES

A stability study was initiated in January 1972, 2 months after the rat study (E-77/78) had begun. The objective of the study

was to evaluate the stability of SC-19192(DKP) when mixed with Rockland mouse/rat diet and held at room temperature (73 degrees F.). Two concentrations of diet mixture were tested: 3.0 % and 6.0% DKP. A preliminary analysis was performed on 1-31-72 to test the analytical method (T.L.C.), and recovery of DKP. Assays were performed at one-week intervals on 2-16-72, 2-23-72, 3-1-72, 3-8-72, 3-15-72, 3-23-72, and 3-29-72. Copies of all analytical reports were obtained and are attached to this report, along with a copy of the protocol. (See exhibits #24-27).

The titration method of DKP analysis was used initially, along with the TLC method. The titration method was discontinued after the 1-week analysis on 2-23-72. Thin layer chromotography was used thereafter. It should be noted that the titration method was the only reliable quantification method for DKP analysis.

Page #54 of laboratory notebook #51 (See Exhibit #26) indicated (from the photograph) that there was something in the basal diet itself producing a spot on the TLC plate which had an Rf. value corresponding to DKP. This would make quantification of DKP by this method difficult.

Some of the photographs of the TLC plates attached to laboratory notebook #51 showed no DKP reference standards. The analysis described on pages #69-72 did use a DKP standard but those on pages #88-89, #106-107, #144-145, and #284-285 showed no reference standard. (See Exhibit #26)

Only one solvent system was used for development of TLC plates throughout the study, even though it was apparent that some material in the basal diet was producing a spot on the TLC plate with an Rf. value corresponding to DKP. With the above method of analysis, only materials reacting with the potassium iodine starch reagent would be detected. Another solvent system was available for TLC analysis of DKP (See Exhibit #19) but apparently was not used in the stability study.

It should also be noted that some of the chromatograms showed poor separation (day 28 on pages #144-145, and day 35 on pages #156-157 of notebook #51). (See Exhibit #26)

In general, the data described in the reports of analysis corresponded well with the laboratory notebooks, although the poor chromatograms were not mentioned in the reports of analysis.

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Skokie, Illinois 60076

The level of impurities as indicated by TLC was low; the major impurity, an unknown substance, represented about 2% of the DKP. The remaining impurities were also low, as apparent from the density of the TLC spots compared with the DKP spots, but were not quantified.

A glossary of terms for aspartame and its diketopiperazine is attached as exhibit #9 and copies of specifications for DKP are attached as exhibits #16-18.

No homogeneity tests were performed on any batches of diet mix used in E-77/78, and evidence exists that homogeneity was a problem with the DKP diet mixtures. Two of the stability study assay reports, analytical numbers A7738 and A7739 both dated 2-16-72, contained the statement: "These samples were not homogeneous. They had to be reground before they could be sampled". The assay reports were signed by Barbara Bickford, a Searle analyst.

We examined the laboratory notebook #51 assigned to Barbara Bickford and noted that a B & W polaroid photograph of the non-homogeneous sample in question was attached to page #58 of the notebook. The photograph clearly shows discrete lighter colored particles of diverse size and shape distributed non-uniformly throughout the mixture. These lighter colored particles appear to be distinct from the fairly fine granular nature of the chow itself.

A copy of this photograph was made and is attached to the report as exhibit #29. When questioned about the size of the white square sheet of paper in the photograph (on which the diet mixture was placed) Ms. Bickford and C. Seul both stated that it was 6"x6", when we interviewed them on 6-2-77. When the photograph was enlarged until the sample paper was 6"x6" (actual size) we measured the large particles (which were identified as DKP by Ms. Bickford) and found them to be 4 to 6mm in size.

When we interviewed Ms. Bickford on 6-1 and 6-2-77, she stated that she had nothing to do with the preparation of the diet mixtures. She said that the samples had probably been received from the toxicology lab and stored at room temperature. Her procedure was to weigh out a predetermined amount of the sample, and if not a uniform powder she would re-grind it with a mortar and pestle, and would make a note of this in her lab notebook. We asked Ms. Bickford if she ever reported this lack of homogeneity to Dr. Rao, and she replied that she did not.

We could not determine whether the samples assayed in the stability study were from diet mixtures actually fed to the animals, in spite of the fact that we were told so by some employees.

On 6-2-77, we interviewed Analyst Barbara Bickford and Clifford Seul, who was Mrs. Bickford's supervisor at the time that the stability samples were analyzed (Feb. 16, 1972). Clifford Seul told us that the samples analyzed on 2-16-72 and described on page #58 of laboratory notebook #51, were obtained from the admixture being fed the rats on study, and not a special mixture prepared for the stability study.

On 6-1-77 we interviewed Bart Tangonan, whose duties included observing, weighing, and feeding the animals, and mixing the diet for study E-77/78. Mr. Tangonan did not remember if there were any written instructions for mixing the diets but thought that it was mixed for a specified length of time. He said that if the diet appeared to need more mixing, it was mixed longer. He could not remember anything about the samples obtained for the stability study.

On 6-3-77 we interviewed Tony Martinez who was a supervisor in the Toxicology Laboratory in 1972. He told us that although the analytical report indicated that the sample was submitted by Dr. Rao, actually anyone in the toxicology laboratory could have submitted the sample. According to Mr. Martinez, the normal procedure in such cases was to collect a sample just after mixing compound and diet and then repeat this in four weeks. He could not specifically recall what was done with regard to the stability study in question, and could not remember whether the samples had been taken from the diets being fed the animals on study P.T. 988S73 (E-77/78). He did not remember any problems with mixing, but did say that a longer mixing time was required at higher compound concentrations.

A point to be considered, however, is that although the analytical report states that the material analyzed was prepared to contain 3.0 and 6.0% DKP, none of the diets reported to be fed contained these exact amounts of DKP according to the records of food concentration calculations, which were used to prepare the diets for study #E-77/78. (See chart attached as Exhibit #30.) In addition, the stability study protocol (Exhibit #24) specified that the test batches would be 1 kg. in size. If the protocol was followed, the small (1 kg.) test batches would not have been sufficient in size to feed a single dose group of the animals on study. (See Protocol, Exhibit #24)

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Additional evidence of homogeneity problems was revealed when a former Searle employee, Raymond Schroeder, was interviewed by the other FDA team on 6-22-77 concerning teratology studies E-5 and E-89. At that time Mr. Schroeder volunteered the information that homogeneity may have been a problem in the DKP diet mixtures, but not in the aspartame diet mixtures. A follow-up phone call to Mr. Schroeder was made on 7-13-77, and at that time he stated that he observed the DKP diet mixtures being fed to the animals, and that in his opinion, the particles of DKP were large enough to allow the rats to discriminate between the DKP and the basal diet. (See Thomas F. X. Collins memos (2) dated 7-14-77 (attached as Exhibit #31). An interview was arranged for July 18, 1977 between Mr. Schroeder and members of the FDA team investigating study E-77/78. The interview was conducted at [redacted], Mr. Schroeder's current place of employment. Also participating in the interview by means of a conference phone were Thomas F. X. Collins, and Leonard Friedman. Mr. Schroeder stated that he was not certain of the date, or even the year, when he observed the rats being fed DKP diets. He further stated that he could not be absolutely certain that the rats he observed were on study E-77/78. He was not certain about the dose levels of the diets he observed, and could not remember how many times he observed the DKP diets. He estimated that he observed the DKP diets "one or two times". When he was shown an actual-size enlargement of the DKP diet mixture (See Exhibit #29) he stated that to best of his knowledge, the white particles that he observed were not as large as the largest particles in the photo, but may have been similar to the smaller white particles. He said that he may have mentioned the appearance of the DKP diets to Dr. Rao.

Mr. Schroeder seemed reluctant to make any positive statements during this interview. Dr. Collins reminded Mr. Schroeder that he had previously volunteered the information that the DKP diets appeared to be non-homogeneous and that the rats could probably discriminate between the DKP particles and the basal diet. Mr. Schroeder replied that he had had some time to think over his previous statements and now wasn't sure about them. He told us that there must be people at Searle who knew more about the DPK diets than he did. (See memo dated 7-19-77, attached as exhibit #32, which describes our interview with Mr. Schroeder).

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When we arrived at [redacted] on 7-18-77 at approximately 2:40 P.M., we were asked by the receptionist to sign a log book. While signing the log, we noted that a G. D. Searle employee (W. R. Pool) had signed in on 7-15-77. W. R. Pool works in the Toxicology Section (Safety Assessment Division) at Searle Laboratories.

During our interview, we asked Mr. Schroeder if he had been contacted by anyone from Searle during the period from June 22, 1977-July 18,1977. He replied that he had not.

We again interviewed Tony Martinez on 7-19-77, and specifically asked him if he was aware of any homogeneity problems with the DKP diet mixtures fed the rats in study #988S73 (E-77/78). He replied that he was not aware of any problems. We asked whether any samples of DKP had been retained by Searle Laboratories. We were told that a small quantity of DKP remained in the compound file, but that it was a lot other than those used in study E-77/78. Upon request, we were then shown a jar containing 4.9 grams of DKP, lot #TJT-12-32. Its appearance was that of a fine white crystalline material with a tendency to adhere to the sides of the jar. Mr. Martinez said that this was the only lot of DKP remaining at Searle.

We also interviewed Teratologist Alan Mitchell, on 7-19-77. We had previously noticed his name on one of the DKP compound inventory cards, and his name had also been mentioned by Raymond Schroeder, in connection with DKP. Mr. Mitchell stated that he had done two teratology studies with DKP, both with rats, and both in 1972. In one study the DKP was administered I.G. (as a suspension), and the other was a dietary feeding study. Mr. Mitchell told us that he didn't recall any problems with homogeneity in the dietary feeding study. He said he never remixed or reground any DKP diets. He admitted, however, that when he prepared the diet mixtures, he first sifted the DKP through a hand flour sifter.

We attempted to interview a former Searle employee, Dr. Rao, after learning that he still lived in the Chicago area. Dr. Rao had been in charge of the DKP stability study and was the monitor for study E-77/78. After reaching Dr. Rao by telephone on July 25, 1977 he stated that he would like to talk to his attorney before consenting to the interview. We then received a call from his attorney, Mr. John H. Bickley, Jr., who told us that the interview would be of no advantage to his client, and he therefore refused to allow it. A memo

of telephone conversation between J. Bressler and Mr. Bickley is attached as Exhibit #33.

CALCULATING DIET CONCENTRATION & BLENDING OF TREATMENT MIXTURES

There were no batch records to show the quantities of DKP and basal diet weighed, type of mixer used, mixing time, dates, or names of individuals performing the weighing and blending operations. We were told that mixing was performed In a Hobart mixer, and that mixing times were about 10 minutes. There was no evidence that any tests had been done to determine the blending characteristics of the mixer, or to validate the 10 minute mix time. Fresh batches were mixed on a weekly, bi-weekly, or monthly basis, and batch size ranged from 6 kilograms to 28 kilograms during the study.

The concentration of DKP in the basal diet was calculated by the Math-Stat Department on a weekly, bi-weekly, or monthly basis (based on the food consumption for the previous time period), and submitted to the Path-Tox Department as a Food Concentration Prediction record. The concentration was expressed as grams of DKP per kilogram of basal diet. The Path-Tox Department Personnel then multiplied the grams of compound indicated on the prediction record by the number of kilograms of diet mix needed to arrive at the proper quantities of DKP and basal diet to be blended. The concentrations were calculated to yield the proper dosage levels of 0.75, 1.5, and 3.0 grams of DKP per kilograms of body weight per day, for the low, medium, and high dose groups. (Copies of Diet Calculation Records are attached as Exhibit #34). At the end of each treatment period, the remaining treatment mixtures were discarded and fresh batches were made.

No reserve sample of either the DKP or the DKP/diet mixtures used in this study were retained according to Searle.

DKP was withdrawn from stock by means of a compound inventory card, which was filled out by the person requesting the material. Tony Martinez was the person that usually requested DKP for use in study E-77/78. We examined eighteen (18) compound inventory cards which accounted for 177.0 kg of DKP withdrawn from stock. According to our calculations a total of 152.81 kg of DKP would have been necessary to

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achieve the diet concentrations and batch sizes that were reportedly used in the study. A total of 230.0 kg of DKP was manufactured by Searle Chemist Jack Drogt. Following are tables showing the quantities of DKP manufactured, calculated quantity required for the study, and quantities withdrawn from stock.

QUANTITIES MANUFACTURED

Lot #.	Quantity (After Milling)
1R	
2R	
3R	
4R	
5R	
6R	
7R	
TOTAL	

CALCULATED QUANTITIES REQUIRED FOR THE STUDY

Dose Group	Calculated Quantity Required
Low Dose Males	kg
Mid Dose Males	kg
High Dose Males	kg
Low Does Females	kg
Mid Dose Females	g
High Dose Females	g
TOTAL	g

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QUANTITIES WITHDRAWN FROM STOCK (FROM COMPOUND INVENTORY CARDS)

Date Withdrawn From Stock	Quantity	Lot #:
10/29/71	kg	1R
1/4/72	kg	1R
2/28/72	kg	4R
3/11/72	kg	3R
3/29/72	kg	2R
9/11/72	kg	3R
10/10/72	kg	2R
*	kg	2R
12/1/72	kg	3R
*	kg	4R
12/27/72	kg	5R
*	kg	2R
1/25/73	kg	5R
3/22/73	kg	6R
4/18/73	kg	5R
7/10/73	)5 kg	8R
8/10/73	)5 kg	6R
9/7/73	kg	6R
11/2/73	kg	7R
TOTAL	kg	

* These three cards were not signed or dated.

It should be noted that only two of the 18 compound inventory cards specified that the DKP withdrawn from stock was to be used in study E-77/78 (PT 988S73). Thirteen of the cards list "Toxicity" or "Toxicology" as the reason for withdrawl. Three of the cards have no entries at all, except for the word "empty". (Copies of the compound inventory cards are attached as Exhibit #28).

The total quantity withdrawn from stock is kg in excess of the amount necessary to achieve the diet concentrations used in the study. (Based on the diet calculation records attached as Exhibit #34, which we used to construct the diet calculation summary table attached as Exhibit #30).

It is not known whether any of the kg of DKP accounted for on the 18 compound inventory cards was withdrawn for use in studies other than E-77/78. We could find no other records to verify the amount of DKP withdrawn for, or used in this study.

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ANIMALS UNDER TEST

Three hundred and sixty weanling albino rats, CD strain, 180 of each sex, were used. The rats were 21 days old when received from the . Copies of shipping labels were obtained, and are attached as Exhibit #10.

The rats were housed individually in wire cages and were given a one-week acclimation period before being placed on treatment at the age of four weeks.

Rockland Rat/House Diet (complete), , was fed for the first 62 weeks, and Rat Chow was used from week 63 until the study was terminated at 114 weeks.

The animals were housed in air conditioned rooms maintained at 72 degrees F, with artificial fluorescent lighting at 12 hours per day exposure.

The rats were divided into 12 housing groups, (6 groups per sex), 30 rats in each housing group. Initiation of treatment was staggered over a 2 week period, beginning 11/8/71.

Each housing group was composed of dosage groups as follows:

Treatment Group	Dosage gm/kg/day	Multiples of Estimated Daily Human Dosage	No. of Rats Per Housing Group Male	Female	Total Rats
Control					
Low					
Medium					
High					

RANDOMIZATION OF ANIMALS

Computer-generated randomization tables were used for assigning the dose and housing groups (copies of these tables were obtained and are attached as Exhibit #6). Each housing group consisted of 30 animals (12 controls, 6 low, 6 medium, and

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6 high dose). Each animal was assigned a letter to designate the housing group (A through M), a cage number (1 through 30), a letter to indicate dose group (C,L,M, & H), and a letter to indicate sex (M or F). For example, animal A30CM would be a control male, in housing group A, occupying cage number 30 (Exhibit #69).

Each rack (30 animals) contained a random distribution of control and treated animals. An example of a typical housing group is shown in the diagram attached as Exhibit #7.

The specific problems of feeding animals housed in the above manner were discussed in the report generated by the task force investigation of Aspartame in 1975/1976. We will re-iterate them here:

Housing experimental animals in this manner (controls, low, medium, & high dose animals randomly distributed on the same rack) would greatly increase the chance of administering the wrong diet to the animals. The chance of error was compounded by the method used to feed the animals which was as follows: At the specified intervals, the animals were weighed, and the empty food jars were removed, weighed and new food jars placed in the cages. The new (filled) food jars were placed on a mobile cart in rows corresponding to dose group (See Exhibit #8). The cart was wheeled to the Intec Unit and placed up ag inst it with the rows of high dose jars farthest away from the operator. The operator started from the upper left corner of the housing rack, (See Exhibit #7), removed the mylar card from the cage and inserted it into the Intec Unit. This printed out the animal's identification number. A color coded card for dose level, also bearing the animal number, remained on the cage. The technician then opened the cage, removed the animal, placed it on the scale pan, pushed the button to register the weight and returned the animal to its cage. He then removed the empty food jar, placed it on the scale and pushed the button to record the empty feeder weight. The empty jar was placed on another mobile cart provided for that purpose. The new (filled) jar was selected from the appropriate row according to dose level (Exhibit #8), placed on the scale, weight recorded, and the jar placed in the cage. The Card was then removed from the Unit and replaced on the cage.

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This procedure was repeated for all cages proceeding from left to right. It is important to note that none of the food jars were identified in any manner, as to animal number or dose level. The position of the jar on the cart was the only means of identifying the proper dose level.

This procedure was used when concentrations were changed. At other times, the feeder jars were weighed; filled with the appropriate diet/dose, weighed and replaced in the cage. If a feed container was almost empty, or contained feces, it would be replaced with a new container.

IDENTIFICATION OF ANIMALS

Ear clips or other methods of uniquely identifying each animal were used. Animals were individually housed (one animal per cage) and a color-coded card containing the cage number, compound number, project number (Path-Tox No.), and dose level, was attached to each cage. When an animal died during the study, the color-coded identification card was removed from the cage and accompanied the animal to the necropsy laboratory.

Also attached to each cage was a Mylar Card which identified the animals for the Computer System.

At the time of death or sacrifice the animals were assigned a pathology number which was used to identify the animal during tissue processing, and on all records pertaining to pathology. The pathology number was a five-digit sequential number assigned by the pathology department. An example of a pathology number is 94.893. The number was etched on all slides as a permanent identification.

All animals that died during the study were assigned an additional number called a "Master Number". The master numbers denote the chronological order in which the animals died during the study, by dose group. For example, CM38 would be the 38th Control Male to die during the study. Master numbers were noted only on the gross pathology sheets and not recorded for all animals. Four animals that died during the study had no master numbers. We were not able to locate any other records providing the missing numbers. Also, two of the master numbers (CM28 and CM29) were out of sequence.

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A chart was made by the FDA team, which shows the animal number (cage #), pathology number, master number, and the complete pathology history of each animal. The chart is organized by dose group, and is attached as Exhibit #35.

ANTE-MORTEM OBSERVATIONS

"Observations For Drug Effects" records are attached as exhibits #70 & 71. These records were completed on a weekly basis for the first four weeks, every two weeks through week 12, and every 4 weeks thereafter. Each record lists the animals in a specific housing group, and entries are made for the following parameters: appearance and awareness, rales, eyes, motor activity sensory loss, urine/feces, appetite/thirst, and tissue masses/lesions. There is also a space for notes, and masses are routinely described on the reverse side of the sheet. The top of the sheet has blocks for entering the date of observations, number of weeks on treatment, and signature or initials of the person making the observations. It was noted that many of the observation records were not signed or initialed. Following is a tabulation of the numbers of records in which the person making the observations is not identified.

HOUSING GROUP	NO. OF RECORDS NOT SIGNED OR INITIALED	
A	6	(wks 80, 92, 84, 88, 93, 94)
B	6	(wks 80, 84, 88, 92, 93, 96)
C	5	(wks 80, 84, 88, 92, 96)
D	7	(wks 79, 80, 84, 88, 92, 94, 96)
E	6	(wks 76, 80, 84, 88, 92, 96)
F	9	(wks 76, 77, 80, 81, 84, 88, 92, 93, 96)
G	5	(wks 80, 84, 88, 92, 96)
H	11	(wks 1, 68, 76, 80, 81, 83, 84, 88, 92, 95, 96)
J	6	(wks 76, 80, 84, 88, 92, 96)
K	8	(wks 71, 76, 80, 84, 88, 92, 93, 96)
L	5	(wks 76, 80, 84, 92, 96)
M	5	(wks 76, 80, 84, 92, 96)
TOTAL:	79	records not signed or initialed

In addition to the lack of signatures, it was noted that many of the records were not originals, but appeared to be xerox copies of the originals. Surprisingly, some of the xerox copies had

"original" initials. It was obvious that the initials had been placed on these sheets sometime after the sheets had been filled out, and after they had been copied.

Some examples of discrepancies of this type are as follows:

1.) In housing group A, 26 of the 39 observation records were xerox copies with original initials.
2.) In housing group B, 27 of the 43 observation records were xerox copies with original initials.
3.) The record for week 76 of housing group A was a xerox copy but the date, initials, and week are all original.
4.) For week 96 of housing group K, both an original and xerox copy of the observation record are present. The xerox copy has original initials and a "B" entered in the "tissue masses and lesions" column. There is also an entry in the "notes" column for rat #K25CF. The original record also has a "B" entered in the "notes" column. All of the above entries had obviously been made sometime after the original record had been completed and the xerox copy made.
5.) A record dated 4-27-73 for housing group M does not have the date entered. The observation sheets for animal A23LM indicate that this animal was alive through week 88. No observations were made for this animal on sheets covering weeks 92, 96, 100, and 104, indicating that the animal was dead. The record for week 108, however, shows that the animal is alive, with motor activity, appetite, and thirst. The record for week 112 again shows that the animal is dead.

In addition to the discrepancy noted above, there is also an obvious error in the dating of these records; the observation sheet for week 92 is dated June 13, 1973, and the observation sheet for week 88 is dated July 16, 1973.

Ante-mortem observations were also made on other types of records. A volume entitled "Tissue Masses and Deaths" (exhibit #65) has a record of the date that each animal died during the study. The deaths are recorded in two different ways in this volume. One record has a chronological list of deaths, and another record has a list of deaths organized by housing

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group. This volume also has a "Palpation Record", which describes each tissue mass, and lists the date that it was initially detected.

It was noted that many of the animals in the sequential record of deaths were listed out of sequence. Following is a tabulation of the animals that were out of sequence:

(* indicates animal is listed out sequence.)

ANIMAL NO.	DATE FOUND DEAD	NO. OF DAYS ON TREATMENT
E6HM	8-12-73	640
E24HM	8-16-73	640
G10LM	*8-14-73	638
L11CM	5-6-73	535
C17CM	*5-4-73	542
A14MM	5-21-73	560
A24HM	10-15-73	707
C14HM	*10-27-73	718
E22CM	*10-19-73	707
E29LM	10-25-73	714
A4CM	10-26-73	718
E26CM	11-15-73	735
C12LM	*11-14-73	736
L20CM	11-19-73	732
A11CM	11-25-73	748
A18CM	*11-18-73	741
J19MM	12-10-73	754
F25HF	6-10-73	576
D21CF	*6-9-73	577
H6CF	6-17-73	579
M12CF	*6-16-73	575
F18CF	7-22-73	615
H22LF	*8-20-73	641
D25MF	7-25-73	623
G2LM	10-5-73	689
G7CM	*6-14-73	567

The log of animal deaths in the "tissue masses and deaths" book was considered the primary data. Animals were allegedly recorded in this book as they died. When the above discrepancies were pointed out to Searle personnel, we were told by Tony Martinez on 4-28-77 that this log was compiled from the body and feeder weight data. When it was pointed out that the exact date of death could not be determined from the body and feeder weight data, or from the observation records, we were told that the primary record of animal deaths was the log organized by Housing Group in the "Tissue Masses and Deaths" book. We were told that the chronological log of animal deaths was made by transferring the data from the log organized by housing group, and therefore the animals being recorded out of sequence was not significant.

We then pointed out that the chronological record of deaths also contained the date fixed "in toto" and the date autopsied, neither of which were found on the log organized by housing group. If the data was transferred from one record to the other, we wondered where the "date fixed in toto" and "date autopsied" came from. We posed this question to Searle personnel and we were finally told on 4-29-77 that the "fixed in toto" and "date autopsied" columns on the chronological record of deaths was considered to be primary data, although it was prepared simultaneously with the pathology sheets, which contained the same data.

Another source of ante-mortem data was the ophthalmoscopic examination record. Dr. Youkilis performed the eye examinations at periodic intervals and recorded his findings on the ophthalmoscopic record sheets. These eye exam sheets were eventually attached to the pathology records, and the results of the eye exam was incorporated into the Clinical History of the animal on the pathology sheet.

During our review of the pathology records we noted that there were no eye exam sheets for 15 animals, all of which had been included in the individual Ophthalmoscopic Findings in the submission to FDA (Vol. 1, table 1, pages 122-133). All of these missing eye exam sheets were for animals that had died during the study. On 6-30-77 we interviewed Donna Helms, who told us that she would make an attempt to find the missing records. We advised her that Dr. R. Stejskal had told us on 6-29-77 that not all of the eye exam sheets were attached to the pathology records, and that there may be another file of eye exam sheets somewhere.

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On 7-1-77 Donna Helms reported that she had found the missing eye exam sheets in the K-1 File Room in K-Building. After reviewing these records we found that a few discrepancies still existed. They are as follows:

1.) It appears that animal J3CM on page 125, Vol. 1 of the submission to FDA is in error. We could find no records to substantiate the listed corneal scar and haziness for this animal. Also, the observation records indicate that J3CM was still alive at week 96, while the table on p. 125 indicates that J3CM died at week 78. It appears that the correct animal on page 125 should be J2CM and not J3CM.

2.) We found eye exam sheets for H26MF and J29CM, yet the findings were not reported in the submission to FDA.

3.) There seems to be a discrepancy between G15CM and G12CM. The pathology sheets for both of these animals report the identical ophthalmosocpic finding, yet there is no eye exam sheet for G12CM, and only the finding for G16CM was reported in the submission to FDA (Vol. 1, p. 125).

During our data review, we found an internal memo from Dr. Youkilis to K. S. Rao, dated 4-28-74. The text of this memo is as follows: "

A copy of the above memo is included in exhibit #72. Dr. Rao and Dr. Youkilis are no longer employed by Searle.

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When an animal spilled an excessive amount of food, this was noted on the observation records by means of an asterisk in the "appetite/thirst" column. The asterisk was also used to denote food spillage on the teletype sheets for body/feeder weight data. The amount of food spillage was not quantitatively determined by the technicians assigned to observe, feed and weigh the animals, but we were told that they made an effort to return spilled food to the food cups whenever possible. We were also told that food consumption data for those rats marked with an asterisk on the body/feeder weight sheets was not used in Searle's statistical analysis of the data.

The "palpation record" in the "Tissue Masses and Deaths" volume shows that tissue masses were sometimes excised from the animals. The record indicates that a tissue mass measuring 1.5x 1.0cm was excised from animal B3L8F on 2-10-72. The record also shows that a "skin incision over mass" was performed on animals C22LM and G25LM on Feb. 10, 1972.

DOSAGE, BODY WEIGHT AND FOOD CONSUMPTION

DKP levels for the feeding study were multiples of 100, 200 and 400 times the estimated human dose. The levels in g DKP/kg body weight/day were 0,0.75, 1.5 and 3.0 for the control, low, medium and high treatment groups, respectively. The doses were mixed in the diet as described in Calculating Diet Concentration and Blending of Treatment Mixtures.

Individual body weights were recorded weekly for the first four weeks, once every two weeks for the next eight weeks and once every four weeks thereafter. The amount of food consumed was measured every week. An automated weighing system was employed consisting of an Intec balance and a teletype machine. The teletype produces a typewritten sheet and a machine-readable punched paper tape. All the typewritten sheets for the study were available. Xerox copies of these sheets were taken to the Division of Mathematics and Technical Operations Staff of the Bureau of Foods where the data were converted to machine-readable form and calculated by a computer program designed by Dennis Wilson, Division of Mathematics.

In designing the computer program it was necessary to make certain assumptions on the handling of the data. One assumption concerned missing data, e.g. the empty feed cups weights were missing for the" D" housing group at the 12th week. Dr. George Clay, Group Leader, CNS Pharmacology, Searle and scientific co-ordinator for the FDA team, was unable to determine whether

these animals were omitted from the food consumption calculations for that week, or whether the data for these animals from the 11th and 13th weeks were averaged and the average substituted for the missing data. Employees of Searle's Math-Stat Department who had worked on the program for this experiment are no longer with the company. Dr. Clay calculated a few of the figures from the 11th and 13th weeks and stated that it appeared that the data had been averaged. For the FDA recalculation it was chosen to omit the animals with the missing weights from the calculations. In several instances (For example, C group males, mid and high levels for the 13th week; A group males, high level for the 99th week) the dietary concentration shown on the weight sheets did not agree with the concentration listed for that level in the other housing groups. Dr. Clay assured us that all the animals of the same sex in a given experimental group received the same dose for the same week on the experiment. He also assured us that the Searle computer program did not pick up the doses from the weight sheets. In the FDA program, the dietary concentrations were taken from the diet calculation sheets (Exhibit #34). Certain animals on the raw data sheets were marked with an asterisk. Dr. Clay explained that the asterisk indicated spillage and such animals were omitted from the food consumption calculations. This practice was followed in the FDA computer program. In calculating the food consumption (g food eaten/day/ kg body weight) and the dosage (mg test compound/day/kg body weight), the body weight used was the weight at the end of the period under consideration, i.e. the current weight.

In addition to the calculations which were included in the Searle submission, the FDA program included calculation of the actual amount of food ingested, i.e., the total amount of diet ingested minus the test compound, and of the food efficiency (g weight gained/100 g actual food eaten). The food efficiency was calculated in order to determine whether the volume of DKP in the diet (which exceeded 7% of the diet for the high dose males at intervals during the study) was contributing to the body weight depression seen with DKP. This explanation of the body weight depression was discussed by Dr. John H. Rust, a Searle consultant, in a memo dated April 5, 1976 to Dr. R. McConnell; in a memo to the file dated September 30, 1974 by Dr. McConnell; and in a memo to Dr. K.S. Rao dated August 29, 1974 by Dr. G.L. Schoenhard. (Exhibits #36-38).

The average body weights and weight gain (% change/week) from the FDA analysis of the Searle raw data are presented in Table 1 (Exhibit#39) which corresponds to Table 3 of the Searle submission.

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Weights which differ from the Searle submission by one (1) g or more and weight gains by 0.1 percentage point, or more are underlined. Fifteen differences were noted as follows:

Average Body Weight Discrepancies

Days	Sex	Dose Level	Searle Submission	Calculated
280	M	M	591.7	589.2
364	F	L	353.2	345.1
420	M	M	613.2	614.4
700	M	C	595.4	579.3
728	M	C	594.4	597.2
728	F	H	343.1	341.2
784	F	C	453.4	456.9

Percent Weight Gain Discrepancies

Days	Sex	Dose Level	Searle Submission	Calculated
14	M	C	35.11	35.23
21	M	C	22.80	22.69
280	M	M	0.33	0.21
364	F	L	-0.16	0.04
392	F	L	1.13	0.85
728	F	H	0.32	-0.18
756	F	H	-0.14	0.08
805	F	C	-0.04	-0.39

The food intake (in g/day and in g/kg/day) and dosage (in mg/kg/day) from the FDA analysis are presented in Table 2. This table corresponds to Table 4 of the Searle submission. There are numerous discrepancies (in excess of 80) of one (1) gram or greater in the food intake expressed in grams/day. Many of the discrepancies are probably the result of an error in the Searle computer program (see Exhibit #76). Through this error there was a failure to adjust the food intake for the precise number of days between weighings for the individual housing groups. This programming error had been pointed out to Searle by the Task Force but no amendment to the Searle submission was made. There are more than forty discrepancies of 5 or more grams when the food intake is expressed in g/kg/day. The Searle programming error would contribute to discrepancies in this expression of the

food intake. The use of the current body weight in the FDA analysis may also be a contributing factor. Most of the dosage calculations from the FDA program differ from the Searle submission by 10 or more mg. The two factors of the Searle programming error and the use of the current body weight in the FDA analysis would contribute to discrepancies between the FDA analysis and the Searle submission. Despite the discrepancies the FDA analysis shows dosage levels corresponding to the intended levels of 0.75, 1.5 and 3.0 g/kg/day. The test compound would have to be homogeneously mixed into the basal diet in order for these calculated dosage levels to be actually consumed. All discrepancies between the Searle submission and the FDA analysis shown in Tables 1 and 2 are underlined.

Table 3 presents the food efficiency (g gained/100 g actual food consumed) calculated in the FDA analysis. There is no corresponding table in the Searle submission. Tables 1, 2 and 3 and the computer printout of the FDA analysis are Exhibits # 39-42. Statistical analysis of the body weight and food consumption data was made and is shown as exhibit #73.

ORGAN WEIGHTS

Organ Weights were entered on the gross pathology sheets at the time of autopsy. We compared all of the individual organ weights on appendix table 5 in the submission to FDA (Vol 1, pp. 222-226) with the original data on the gross pathology sheets. A total of eleven (11) errors were noted in transcribing the raw data from the pathology sheets, to the tables in the submission to FDA.

The errors are tabulated below:

Animal No.	Organ	Wt. Shown In Submission	Wt. Recorded on Original Pathology Sheet
A12CM	Kidneys	3.75 G	3.45 G
L28LM	Ven. Prostrate	747 mg.	474.7 mg.
C01MM	Kidneys	9.40 G	9.219 G.
CO2HM	Kidneys	1.46 G	4.259 G
E14HM	Kidneys	11.74 G	4.746 G
J12HM	Pituitary	3.0 mg.	3.3 mg.
J30HM	Ven. Prostrate	444 mg.	444.8 mg.
F17CF	Ovaries	36.7 mg.	233.5 & 36.7 mg.
H30CF	Liver	9.4 G	9.493 G
B20HF	Uterus	1115 mg.	1155 mg.
K11HF	Adrenals	799.1 mg.	797.1 mg.

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Copies of the applicable pages of the submission, appendix table 5, with errors indicated, are attached along with copies of the gross pathology sheets documenting the errors. (See exhibit #83)

DISEASES

The submission to FDA (Vol. 1, P. 10) reported that an unidentified infectious disease spread among the animals between 12 and 14 weeks of treatment, and that a second unidentified infectious disease occurred in high incidence between 48 and 52 weeks of treatment. In both cases, the control and treated rats were reportedly affected with equal frequency and severity. The same page of the submission also stated that over a period of two weeks, a total of 17 animals (8 control, 3 low dose, 4 medium dose, and 2 high dose) died. A memorandum dated October 31, 1972, written by Dr. K.S. Rao, and attached to the study protocol, reported that 10 animals had died in 4 days, beginning October 26, 1972, and that more animals were morbid. Dr. Rao reported that the primary antemortem symptom observed was inappetance and labored respiration. Postmortem examination of dead animals revealed primary lesions in the lungs, and lungs exhibited patchy pneumonia, according to Dr. Rao. The memo indicates that Dr. Rao intended to administer 10,000 units of penicillin G, intramuscularly, to all the animals 2 or 3 times per day beginning 10/30/72. A copy of Dr. Rao's memo is attached to the protocol (See Exhibit #77, Section I).

The submission to FDA (Vol. 1, P. 10) stated that, "to prevent further loss of animals, all morbid rats were injected IM with 20,000 units of potassium penicillin G daily for 4-8 days."

A review of the injection records (attached to Vol. A of Exhibit #75) showed that some animals were treated between approximately 51 and 60 weeks, and in one instance, a high dose animal, B3HF, received at least 10 injections. In addition, some animals received 30,000 units per day (10,000 units 3 times per day) rather than the 20,000 units reported in the submission.

The records also indicated that penicillin was administered to four rats beginning on May 16, 1973, and continued daily through May 28, 1973. This third occurrence of infectious disease and penicillin administration was not reported in the submission to FDA.

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SURVIVAL

An attempt was made to construct a Survival Table using data from the "Tissue Masses and Deaths" book.

We were unable to determine the exact method used in constructing the table in the FDA submission. There was some survival data in the "Tissue Masses and Deaths" book (Exhibit 65), but this only extended through week 109 and consisted solely of running totals. According to Tony Martinez deaths purportedly were initially recorded in any one of the following documents:

(1) Body/Feeder Weight Sheets
(2) Autopsy/Pathology Sheets
(3) Observation Sheets
(4) Palpable Mass Sheet

He said that animals found dead at feeding/observation intervals were usually recorded on the Observation, or Body/Feeder Weight Sheets. At other times, the death was recorded on a "scrap" of paper and then later transcribed to one of the documents. The term "scrap of paper" was used by Searle personnel both during the Task Force and current investigations. No notebooks containing observations or deaths ever surfaced during either investigation. Animals killed "in extremis" were recorded on Autopsy sheets. The least likely source for original death recording would be the Body/Feeder Weight Sheets.

Dates of death sometimes differed on the various records, making it impossible to determine which one was correct. A survival table was finally constructed for weeks 40-115, using the Body/Feeder weight teletype (hard copy) sheets and dates on which animals no longer appeared as a base (Exhibit 68). In this manner, the number of days on study was calculated for each animal (Exhibit 66). Using starting dates for each group, a calendar was made to encompass the entire duration of the study (Exhibit 67). Toward the end of the study, some feedings/observations were made at intervals such as 109 3/7, 110 6/7 and 111 6/7 weeks, so some differences are anticipated between this table and the one in the FDA submission. However, the final number of animals in each dosage group and sex do coincide. The table constructed for this report was on a weekly basis; that in the submission covering only weeks 40, 46, 52, 60, 68, 76, 84, 88, 92, 96, 100, 104, 108 and 115.

A Life Table Analysis was performed from the Survival Table by Dennis Wilson, Department of Mathematics, Bureau of Foods (Exhibit #73). The female control population differed from the high level population $p < 0.05$. The male control population differed from both the medium and high dose levels ($p < 0.05$ in both cases). In all cases, the differences are due to the higher mortality level of controls.

CLINICAL LABORATORY ANALYSIS

A. Clinical laboratory procedures.

Hematologic, clinical chemical and urinalysis examinations are described on pages 5-7 of Volume I of the submission. The same rats were employed for all clinical laboratory examinations throughout the study. In cases where one of these rats died during the study, another rat chosen from a corresponding group was substituted.

The following hematology parameters were measured at treatment days 42,92,189,364,547 and 734: hematocrit, hemoglobin, total RBC, total WBC, differential WBC, and prothrombin time.

The following clinical chemistry (serum) measurements were made: pyruvic transaminase (days 42,92,189,364,547,736), glutamic oxaloacetic transaminase (days 41,92,189,364,547 and 734), alkaline phosphatase (days 42,92,189,364,547,734), total bilirubin (days 42,92,189,364,547,734), blood (serum) urea nitrogen (days 42,92,189,364), total cholesterol (days 42, 92,189,364,734), L-phenylalanine (days 42,92,189,364,547,734) sodium (day 734), potassium (day 734), calcium (day 734), protein electrophoresis (day 734).

The following urinalysis (2 hour collection) measurements were made at days 42,92,190,364,547, & 734: specific gravity, pH, occult blood, protein, bilirubin, microscopic on sediment, and phenylketones; glucose and ketones were determined at days 42,92,190,364, & 734; urobilinogen was measured at day 42,190, 364 and 547.

We noted that some of the data sheets for urinalysis had erroneously labeled the phenylketones test values as "phenylalanine" (see exhibit #84).

Some cholesterol and BUN determinations were carried out which were not described in the submission to FDA. They were as follows:

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1) Serum cholesterol determinations were done at days 796 & 798 (terminal bleeding), but not included in the submission to FDA.

The protocol indicated that clinical chemistry determinations, including serum cholesterol, were to have been performed at termination. The submission to FDA (Vol. 1 p. 286) reported a significant decrease in serum cholesterol that was more perceptible towards the end of the study, and may have been related to compound administration. Therefore, the omitted data may have been important. (Copies of these data were obtained and are attached as exhibit # 77, Section V).

2) BUN determinations were done at day 546 but not reported in the submission to FDA (see exhibit #77 Section V).

3) Serum cholesterols were also done on day 546 and not reported in the submission (see exhibit #77). These determinations were only done for females, and only for a few animals, reportedly due to insufficient quantity of sample.

4) BUN's were also done on day 735 and not reported in the submission. This data was not complete for all animals at day 735.

5) Additional animals (other than those designated) were bled at the regularly scheduled times and determinations were made. These determinations were not reported and we could not determine why the animals were bled. (See Exhibit #77)

B. A list of persons involved with lab analysis along with their responsibilities and duties is as follows:

1) Judith E. [illegible]chmal - Nov. 1971 to present, Supv., Clinical Chemistry section of Bioanalytical laboratory.

2) Judith A. Beauchamp - Supervisor, Hematology Laboratory, April 19[illegible] to present.

3) Denise Perkins - Supervisor Hematology laboratory until April, 1977 (no longer employed by Searle).

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Bart Tangonan
Tony Martinez
David Kie
Robert Spaet

The above four persons in the toxicology department were involved with assembling data for clinical chemistry and hematology determinations for April 1973 to Feb. 1974.

Joyce Schulmann - performed urinalysis and hematology determinations from April 1973 to Feb. 1974.

Philip Huellner - Technician in Path-Tox Dept. July 1970 till end of study.

Janet Praal - Technician, prepared individual work sheets for urinalysis. No longer employed by Searle.

C. The following employees were interviewed regarding clinical lab procedures, and methods for recording clinical lab. data.

1) Bart Tangonan on 6/1/77 regarding the recording of data.

2) Judith Beauchamp, on 6/2/77 regarding hematology and urinalysis.

3) Judith Schmal, on 6/2/77, 6/7/77, and 7/29/77 regarding clinical chemistry.

4) Tony Martinez, on 6/3/77 regarding urine and blood collection, and recording of data.

5) Jane Drury, on 6/7/77 regarding electrophoresis.

Accounts of these interviews are attached as exhibits #47-54.

D. Other Documents and Procedures Used to Authenticate Clinical Laboratory Data values in Submission were as follows:

1) One loose leaf volume entitled "SC-19192: 104 Week Oral Toxicity Study In The Rat. PT - 988S73 Protocols, Organ Weights, Dosage, Hematology, Urinalysis, Blood Chemistry, Protein Electrophoresis." The volume was subdivided into sections according to the above parameters. The indivi-

dual pages (see Exhibit #77, Section IX for example) are composed of forms containing the appropriate measurements and units printed on the left side of the page onto which data on "sticky back sheets" corresponding to each of these measurements were pasted in columns representing the various time periods. These pages, in addition to other information, were headed by the identifying number of the rat for which the measurements were made. The information on the sticky back sheets (see Exhibit #77, Sec. IX) was copied (hand written) from laboratory notebooks, sheets, Autoanalyzer Charts, teletype sheets (on line data generated by analytical instruments) or computer printouts (containing raw and calculated data resulting from on line or off line input data from instruments) by individuals of General Toxicology Section (see interview with Bart Tangonan). Many of the pages were initialed "BRT" (apparently by Bart R. Tangonan). Most of the final values transcribed into the sticky back sheets resulted apparently from calculations made directly by the analytical instruments or by external computer using the appropriate stored equations and data for the reference standards.

2) Since the values appearing in the volume referred to in the above section were copied from other sources, an attempt was made to verify these values by examining the information in these sources. No attempt was made to recover the teletype sheets, or computer printouts which we were told were no longer available or could not be recovered (see interview with Judith R. Schmal, Exhibit #54). All laboratory notebooks that might contain the original data were requested. Notebooks dated prior to the dates of the DRP study were excluded. The appropriate laboratory notebooks were then identified by BA numbers which were listed on the top sections of the sticky back sheets included in the volume referred to above. Examination of these few laboratory notebooks revealed only a very small amount of data which could be used for additional verification of the values in the submission. It was necessary to obtain the consultation of Judith Schmal to clarify the system used to relate the values in these books with the corresponding rat and period of time of bleeding. The following notebooks, as designated by information on the front covers, were examined:

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1) Lab. notebook #N-26375 (hematology), 25 June 71 to 1/21/72.

2) Lab notebook #127133 (phenylalanine), 10/8/71 to 4/21/72.

3) *Lab notebook #113239 (cholesterol), dated 5/1/72.

4) *Lab notebook #17, BA #0007118926 (SGOT), 12/27/71 to 2/25/72.

5) Lab notebook #126472 (phenylalanine), 4/21/72 to 6/8/72.

6) Blue Book #1591, identified "JF VON - 70" (hematology).

7) Columnar book #21, identified "JF VON 27" (Differential cell counts).

8) Spiral notebook identified "JABEA-8" (coagulation/ prothrombin) dated 7/23/71.

9) *Spiral notebook #16, (SGOT), 8/27/71 to 12/16/71.

*Those books (3,4, & 9 above) marked with an asterisk provided us with no useable data, because a formula or standard curve (no longer available) was necessary to convert the data.

Copies of the applicable pages from all of the above notebooks were obtained, and are included in exhibit #77.

The following data were cross checked against available data from original entries (in addition to being checked against transcribed data on "sticky back sheets" in bound volume)

1. Hematology - Erythrocytes:
   Treatment days 42,91,364 & 546, Males and Females.

2. Hematology - Leucocytes, WBC:
   Treatment Days 42,91,364, & 546 Males and Females.

3. Hematology - Leucocytes, Differential:
   Treatment Days 42,91,189,364, & 546, Males and Females.

4. Hematology - Coagulogram, Prothrombin Time:
   Treatment Days 42 & 91, Males and Females.

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5. Phenylalanine:
Treatment Days 42,189 Males and Females, Day 91 Males.

E. Discrepancies were found between the clinical laboratory methods described on pages 5-7 of submission Volume 1 (referenced on page 120) and those actually carried out. These discrepancies were documented by the interviews described in Section C and in a document (Exhibit #77, Section II) voluntarily submitted by Judith Schmal, June 7, 1977 in response to requests for clarification of the clinical chemistry procedures as they were actually conducted in regard to analytical methology instrumentation, and processing and recording of data.

1) Glutamic Pyruvic Transaminase.

Reference: Russell, C.D. and Cotlove, E. (1971). Clin. Chem. 17; 1114

The reference describes a coupled reaction U.V. assay for serum glutamic oxaloacetic transaminase in which malic dehydrogenase is used.

As described by Judith R. Schmal (June 7, 1977) glutamic pyruvic transaminase was assayed by a method adapted from Sigma Kit Technical bulletin #410 - U.V. using lactic acid dehydrogenase.

2) Glutamic Oxaloacetic Transaminase

Reference: Same as in (1) above.

As described by Judith R. Schmal (June 7, 1977), from November 1971 to March 15, 1972 a manual colorimetric method (Fermco Kit) was used (employing dinitrophenylhydrazine). From March 15, 1972 the method used was adapted from Sigma Kit, Technical bulletin #410 - U.V. using lactic acid dehydrogenase.

3) Blood (serum) urea nitrogen.

Reference: Marsh, (Marsk in Submission) W.H., Fingerhut, B. and Miller, H. (1965). Clin Chem 11, 624.

The referenced method calls for reaction of urea with diacetyl monoxime in the presence of thiosemicarbazide and ferric ions in a relatively weak acid solution.

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As described by Judith R. Schmal (June 7, 1977) the method used from November 1971 to February 1, 1974 was adapted from Fermco Kit Bulletins #20 and 20-1. Urea is hydrolyzed to ammonia and carbonic acid in the presence of urease. Ammonia is detected by the Berthelot reaction to produce indophenol. From February 1, 1974 the "direct serum method" modified from the method of Marsh et al was used.

4) Phenylalanine

Reference: Hill, J.B., Summer, G.K. Pencer, M.W. and Resz N.O. (1965) Clin Chem 11, 541

From Nov. 1971 to about September 1972 there is no documentation in file as to method used. From about September 1972 the method used was a flurometric determination in the presence of ninhydrin and l-leucyl-l-alanine as adapted from McCaman and Rubins. (This is a manual method modified for automation by Hill et al - reference above) (Judith R. Schmal, June 7, 1977)

5) Calcium:

Reference Pybus J., (Pylrus in submission), Feldman, F. J., and Bowers (Borrers in submission) Jr., G. N. (1970) Clin. Chem. 16 (11 in submission), 998.

The referenced method involves the measurement of total calcium in serum by atomic absorption spectrophotometry. As described by Judith R. Schmal (June 7, 1977) from November, 1961 to February 1974 the procedure used was a colorimetric procedure using Corinth dye as adapted from Kingsley and Robnet. From May 21, 1973 the method used was atomic absorption spectrophotometry, as adapted from Pybus et al (reference above)

6) Total Cholesterol

Reference: Levine J. S., Morgenstern, S., and Vlastelica, D. (1968). Automation Anal Chem. pp 25-28, Technicon Symposia 1968.

We were unable to check the above reference because of difficulty up to now in obtaining a copy of the publication, but as shown below two different procedures were employed to measure total serum cholesterol at different times during the study

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(Judith R. Schmal, June 7, 1977). From November 1971 to July 2, 1973 the method involved reacting an isopropanol extract of serum with ferric chloride (modified from Block, Tirret and Levine. From July 2, 1973 the method used was a direct serum method using a modified Lieberman - Burchard reaction.

7) Glucose

Reference: Frings, C.S., Ratliff, C.R. and Dunn, R.T. (1969) Advances in Automated Analysis 1, 73

This reference was not checked (because of difficulty up to now in obtaining a copy of the publication) but as shown below two different procedures were employed to measure serum glucose at different times during the study (Judith R. Schmal, June 7, 1977).

From November 1971 to October 16, 1972 the method was a glucose oxidase determination (modified Gertrude Acrow) using protein free filtrates. From October 16, 1972 the method was a direct serum O-Toluidine reaction as modified from Frings, Ratliff and Dunn.

In the case of four of the above parameters (glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, blood urea nitrogen and calcium) different methodology was used during part of the study than was indicated in the submission. For one parameter (phenylalanine), there was no documentation as to the method used for one period of the study and for two other parameters (total cholesterol and glucose), two different methods were used for each of the parameters while only one was referenced in the submission.

Alkaline phosphatase was measured generally as referenced in the submission (McComb, R.B. and Borrers, G.N. (1972). Clin. Chem., 18, 97 in that the method involved measuring the production of p-nitrophenol from p-nitrophenylphosphat However starting July, 1973 there was a "re-optimization of reagent concentrations" (Judith R. Small, June 7, 1977).

The above changes in procedure could conceivably result in differences in the apparent absolute values for the concentration of the substances measured. Changes in the method of conversion of raw data to calculated values as was done in the determination of sodium and potassium by atomic absorption spectrophotometry during different periods of the study, (Judith R. Schmal, June 7, 1977) could also possibly produce differences in final values.

In an interview with Judith Schmal on June 2, 1977, she did state in response to a question that two levels of "Serum Controls" were used in each run to check the method and instruments and that the data was not reported if the values were more than two standard deviations greater than that for the expected values.

No evidence was obtained that any attempts were made to determine whether or not DKP could interfere with any of the clincial laboratory tests conducted. For that matter no information was made available to us as to whether DKP itself or related compounds did appear in the blood or the urine of rats fed diet containing this compound.

Neither, as a result of interviews held or reference to available laboratory notebooks were we able to obtain information helpful in explaining the unusually low values for BUN for the control males at treatment days 189 and 364 and for all treated male groups at treatment day 364. No raw laboratory data in reference to this could be found and may have been recorded on discarded teletype sheets referred to previously. In reference to the low BUN values, Page 29 of the submission contains the following statement: "BUN values for the control males at treatment day 189 were unusually low and may possibly be related to a technical artefact; as a result, the group mean values for all treated males at this interval were significantly higher but, in fact, these values were in the normal range. BUN values both in control and all treated male groups at treatment day 364 were unusually low; this again reflects a possible technical artefact."

F. A total of 21 disparities between individual clinical laboratory analysis values appearing in the submission Volume I and those values appearing in data sheets and/or laboratory notebooks were found (Table 4). Of these, 17 were in hematology, one in clinical chemistry and three in urinalysis. As a result of a discussion with Robert Bost, it was apparent that some of the hematology discrepancies may have resulted from Searle personnel mistaking recorded instrument readings for calculated values. In two cases no value or crossed out values appeared in the laboratory notebooks while values were found entered onto the appropriate places in the data sheets. For animal number A01HM and treatment day 546 four discrepancies (hematocrit, hemoglobin, RBC and WBC) were noted.

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G. Discrepancies Found In Statistical Analysis:

The mean and standard errors for the three dose levels and the controls for the various measurements using the values in the submission Volume I or values noted in the data sheets (where these values differed from those found in the submission) were calculated by the Division of Mathematics, FDA. Also supplied were the results of T-Tests comparing the controls to the treated groups. See memo to Leonard Friedman from Dennis Wilson, dated July 20, 1977 with attached Tables 1 and 2 (Exhibit #87).

A total of 49 disparities were found, which were comprised of 6 means, 23 standard errors and 20 significant differences. As stated in the memo, in all cases where there is a disparity, it appears to be due to differences in the data.

Calculations were also carried out for cholesterol data found in the data sheets but not reported in the submission. As shown in Table 5 the mean values for the medium and high level treated females and the high level treated males were significantly lower then the mean values for the respective controls. To illustrate the possible significance of these changes and disparities between the values calculated by Searle and FDA for the cholesterol data at the other time periods of treatment, table 5 was constructed. Very few disparities are seen between the calculated values obtained by FDA and those in the submission but a fairly consistent trend is seen for treatment related lowering of serum cholesterol, particularly at the two highest dose levels and for the female rats.

Because additional disparities were recently noted in individual hematology values after these statistical computations by FDA were completed (due to the discovery of additional laboratory notebooks), an addendum to this report regarding the statistical disparities reported here will be forthcoming.

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TABLE 5

TOTAL SERUM CHOLESTEROL (MG/DL)

TREATMENT GROUP						
	42	92	189	364	734	798

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GROSS PATHOLOGY

The pathologists reponsible for the microscopic examination (Rudolph Stejskal and Joseph Smith) did not perform the necropsies. Necropsies were performed by Tony Martinez, David Kie and Robert Spaet, with the two pathologists available for consultation.

The submission to FDA (Vol 1, p. 7) reported that "Rats found dead during the study were autopsied immediately whenever possible. In cases where the necropsy could not be performed promptly, the thoracic and abdominal cavitites of dead rats were opened and the entire animal was immersed in neutral buffered formalin fixative for subsequent gross examination and dissection".

Our examination of gross pathology records showed that 98 of the 196 animals that died during the study were fixed in toto and autopsied at some later date, in some cases more than one year later.

A total of 20 animals were excluded from the study due to excessive autolysis. Of these, 17 had been fixed in toto and autopsied at a later date. Following are the twenty animals excluded from the study:

Animal No.	Date Found Dead	Date Autopsied
C21CM	7/3/73	1/11/74
G16CM	9/21/73	1/11/74
G18CM	8/11/73	10/4/73
G26CM	*4/2/73	1/11/74
J2CM	5/21/73	1/11/74
J5CM	10/30/72	11/8/72
L10CM	3/29/73	1/11/74
L15CM	9/9/73	1/11/74
L21CM	4/13/73	1/11/74
L11LM	5/6/73	1/9/74
A14MM	5/21/73	1/9/74
G28MM	1/5/74	1/7/74
J25MM	5/24/73	5/24/73
A3HM	6/17/73	1/9/74
C15HM	1/7/74	1/7/74

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Animal No.	Date Found Dead	Date Autopsied
G13HM	7/25/73	**1/9/74
H24CF	4/29/73	1/11/74
D4HF	7/11/73	7/11/73
D16HF	*4/2/73	1/8/74
F6HF	1/5/74	1/7/74

* Although the date found dead was listed as 4/2/73 on the gross pathology sheet, the "Tissue Masses & Deaths" book listed this date as 4/1/73.

** Although the date found dead was listed as 1/9/74 on the gross pathology sheet, the "Tissue Masses & Deaths" book listed this date as 7/25/73.

The gross pathology sheet for one of the above animals, F6HF, described a tissue mass measuring 5.0 X 4.5X2.5 cm. This tissue mass was first observed on 8/24/73 according to the pathology sheet (Exhibit #79), the observation records (Exhibit #70), and the palpation record in the "Tissue Masses and Deaths" book (Exhibit #65). The submission to FDA (Exhibit #85) reported no tissue mass and the animal was excluded from the study due to marked autolysis.

In addition to the above twenty animals that were excluded from the study, many other animals exhibited marked autolysis. For example, D27LF, M25CF, and H12CF are all described grossly in the submission to FDA as follows; "all organs examined grossly were markedly autolyzed".

Records for approximately 30 animals showed substantial differences between gross observations on pathology sheets, when compared with the individual pathology summaries submitted to FDA. Following is a detailed comparison of ten of these. (Copies of all the gross pathology sheets, and the pathology summaries submitted to FDA are attached as Exhibits #78, #79, and #86).

A2CM

Submission to FDA:

Lung - Focal adhesion
Adrenal - Moderately enlarged

All other organs examined grossly were unremarkable.

Original Pathology Sheet:

Pituitary - Missing
Lung - Left, mid-portion adheres to the medial area of the rib cage by a "fibrous" type of tissue. (Submitted together with relevant portion of rib cage).

Right, post-caval lobe has undergone consolidation. Contains grayish-yellowish nodules measuring 2 X 2 mm. (Entire lung submitted in toto)

Lymph Nodes, Pancreatic - Slightly enlarged

Adrenal - Left, moderately enlarged. Right and left, covered with tiny yellow spots measuring 1.0 X 1.0 - 2.0 X 2.0 mm.
Kidney - Rough surfaces, bilaterally. Left - dark brown spots covering the serosal surface measuring 1.0 X 1.0 mm.

Lymph Nodes, Mesenteric - moderately enlarged.
Mass - previously described on 8/20/73 has since then regressed.
Prostate - Marked atrophy, all lobes
Seminal Vesicles - Marked atrophy, bilaterally

All other organs examined were grossly normal and unremarkable.

M15CP

Submission:

Mammary gland - subcutaneous mass located in mid-thoracic region measuring 7 X 6 X 2.5 cm.

Urinary bladder - papillary growth in the lumen.

All other organs examined grossly were unremarkable.

Original:

Mass #1 - Previously described in the left inguinal region on 2/9/73 has since then regressed.

Masses #2 & 3 - Located in the mid-axillary-cervical regions are all one mass now measuring 7.0 X 6.0 X 2.5 cm and may be described as irregular in shape, multi-nodular, smooth-surfaced, non-glistening,
No Spinal Cord VL - yellowish-purplish in color, non-adherent to the underlying muscle and containing a whitish-yellowish firm tissue within. (Submitted in toto together with remainder of tissue).

Heart - Left Ventricle - dilitation and walls thin.
Spleen - Slightly enlarged.
Liver - Prominent lobular architecture.
Adrenal - Left, slightly enlarged. Right, unremarkable.
Ovary - Right, small cyst measuring 4.0 X 4.0 mm and distended with a clear yellow fluid.

All other organs examined were grossly normal and unremarkable.

G10LM

Submission:

Testis - Marked atrophy, unilaterally.
Kidney - Moderate enlargement, mottled appearance, bilaterally.
Small and large intestine exhibited moderate autolysis, no sections submitted.

All other organs examined were grossly unremarkable.

Original:

Mass which was initially palpated on 2/9/72 (86 days Rx) in the left inguinal area was actually the left testis which ascended and went thru weakened left inguinal ring into the subcutaneous area.

Testis - Left (ascended) appears atrophied (submitted in toto).
Kidney - Moderate, diffuse and uniform enlargement, mottled, bilaterally (submitted in toto).
Small and large intestines are moderately autolyzed (no sections submitted).
Thyroid- Moderately enlarged, bilaterally. A 2 mm in dia., discrete, sl raised, moderately firm yellowish-grey lesion is located in the posterior tip, bilaterally. (Thyroid submitted in toto wrapped in a lens paper).

All other organs examined were grossly normal and unremarkable.

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L11LM

Submission:

Kidney - Mottled appearance
Testis - Marked atrophy, bilaterally
Prostate - Marked atrophy

All other organs examined grossly exhibited marked autolysis.

Original:

Adrenal - Pale yellow, bilaterally
Kidney - Pale yellow, bilaterally, rough-surfaced, bilaterally, moderately autolyzed, bilaterally, tiny spaces in the cortex region measuring about 1 mm in diameter, bilaterally.
Testes - Marked atrophy, bilaterally, marked autolysis, bilaterally.
Prostate - Marked atrophy, all lobes
Seminal Vesicles - Marked atrophy, bilaterally
Spleen - Marked autolysis
Pancreas - Marked autolysis
Stomach - Marked autolysis. Aglandular portion - numerous, tiny, pitted ulcerations measuring 1-4 mm in diameter.
Lymph Nodes, Mesenteric - Marked autolysis
Heart - Wall of left ventricle thin
Lung - Consolidation of all lobes.
Brain - Marked autolysis
Pituitary - Marked autolysis
Liver - Marked autolysis

All other organs examined were grossly normal and unremarkable.

M17LF

Submission:

Pituitary - Marked enlargement.
Adrenal - Markedly enlarged and hyperemic, bilaterally.
Mammary Gland - Mass 1, located subcutaneously in left axillary region, measuring 3 X 3 X 2.5 cm; mass 2, located subcutaneously adjacent to mass 1, measuring 3 X 2 X 1 cm; mass 3, located subcutaneously in the right axillary region, measuring 2.5 X 2 X 1 cm; mass 4,

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located subcutaneously in the left inguinal region, measuring 3 X 1 X 1 cm; mass 5, located subcutaneously in the right inguinal region, measuring 2 X 1.5 X 1 cm.

All other organs examined grossly were unremarkable.

Original

Pit
Adrenal appears markedly hyperemic
Exhibits numerous minute greyish spots on the serosal surface bilaterally. It appears markedly enlarged.

Mass (1) - A 3 X 3 X 2.5 cm spheroidal, multinodular, yellowish white, slightly firm mass located subcutaneously in the left axillary area. Mass non-adherent to the surrounding muscles or tissue (submitted in toto).

Mass (2) - A 2.5 X 2 X 1 cm spheroidal, smooth, yellowish white firm mass located subcutaneously and adjacent to the above described mass (submitted in toto) mass non-adherent to the surrounding muscles or tissues.

Mass (3) - A 2.3 X 2 X 1 cm irregularly shaped, multinodular, yellowish white, firm mass located subcutaneously on the rt. axillary area. Mass non-adherent to the surrounding muscles or tissues (submitted in toto).

Mass (4) - A 3 X 1 X 1 cm elongated, multinodular, yellowish white, firm mass located subcutaneously on the left inguinal area. Mass non-adherent to the surrounding muscles or tissues (submitted in toto).

Mass (5) - A 2 X 1.5 X 1 cm flat, multinodular, yellowish white, firm mass located subcutaneously on the rt. inguinal area. Mass non-adherent to the surrounding muscles or tissues (submitted in toto).

All other organs examined were grossly normal and unremarkable.

CINM

Submission:

Kidney Marked enlargement with yellowish discoloration.
Testis Marked atrophy, bilaterally.

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Tissue mass located subcutaneously in the right inguinal area measuring 2.5 X 2 X 1 cm.

All other organs examined grossly were unremarkable.

Original:

Mass - Previously described on 12/9/72 and located subcutaneously in the right inguinal area now measures 2.5 X 2.0 X 1.0 cm and may be described as smooth-surfaced, purplish-yellowish in color, non-glistening, firm, multi-nodular, non-adherent to the underlying muscle and containing a firm yellowish-whitish tissue. (Submitted in toto together with a portion of the skin and underlying muscle with remainder of tissue).
Heart - Left ventricle has undergone a moderate amount of dilitation. Wall, left ventricle is thin.
Liver - Prominent lobular architecture.
Lung - Right, post-caval lobe-consolidation.
Kidney - Markedly enlarged, yellow and rough-surfaced, bilaterally. Dilitation of the pelvis.
Adrenal - Covered with tiny yellow spots measuring 1 mm in diameter, bilaterally.
Testes - Marked atrophy, bilaterally.

All other organs examined were grossly normal and unremarkable.

Tiss. Trimming - Nodules discovered immediately posterior (2.0 cm) to the pyloric portion of the stomach within the adipose tissue. Nodules may be described as firm, yellowish brownish in color. Non-glistening measuring 1.2 X 1.0 mm to 4.0 X 4.0 mm.

E27MM

Submission:

Lung - Moderate diffuse hyperemia.
Eye - Opaque cornea, bilaterally.

All other organs examined grossly were unremarkable

Original:

Lungs - All lobes exhibit moderate diffuse and uniform hypermia.

Kidney - Moderate autolysis.
Eye - The entire cornea is opaque, bilaterally.
Spleen - Moderately autolyzed.
Stomach- Numerous 1-2 mm. hemorrhagic ulcerations are located on the glandular mucosa. Entire small and large intestines are moderately autolyzed.
Brain & Pituitary - Moderately autolyzed.

All other organs examined were grossly normal and unremarkable.

A1HM

Submission:

All organs examined were grossly unremarkable.

Original:

Testes - Markedly atrophy, bilaterally
Lung, Rt - Middle lobe exhibits a 1 X 1 cm consolidation on the posterior portion.
Liver - All lobes appear olive green otherwise unremarkable.

All other organs examined were grossly normal and unremarkable.

L27HM:

Submission:

Testes - Right, slightly enlarged; left, mild atrophy.

All other organs examined grossly were unremarkable.

Original:

Testes - lt./appears markedly atrophy
rt./appears to be distended with yellowish white substance.
Seminal V- Appears markedly atrophy bilaterally.
Intestine -Large, markedly distended with "gas".

All other organs examined were grossly normal and unremarkable.

P.M. Testes - Also, small black areas are noted within along with the yellowish areas. Black areas measuring 1.0 X 1.0 to 4.0 X 4.0 mm in diameter.

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J30HM

Submission:

Lung - Moderate consolidation of all right lobes.
Testis - Moderate atrophy

All other organs examined grossly were unremarkable.

Original:

Pituitary - Markedly enlarged; slightly hyperemic.
Heart - Left Ventricle has undergone dilitation walls thin.
Lung - Right, anterior, medial and post-caval lobe have undergone consolidation.
Testes - Marked atrophy, bilaterally.

Seminal Vesicle - Marked atrophy, bilaterally.

All other organs examined were grossly normal and unremarkable.

Dr. Stejskal told us that the other pathologist (Dr. Joseph Smith) who made microscopic evaluations of the slides, came from a hospital background (human pathology) and therefore his descriptions and terminology were a little bit different than one would expect from a veterinary pathologist.

MICROSCOPIC PATHOLOGY

We were assisted in our review of the Microscopic Pathology of Study E-77/78 by Charles H. Frith, D.V.M., Ph.D, Director, Pathology Services, NCTR. Dr. Frith arrived on 6/22/77 and spent 3 days with the FDA team. He examined slides for a representative number of animals, the selection of which was made jointly by Dr. Frith and the other members of the FDA team. A Searle Pathologist was not present during Dr. Frith's review of the slides. However, Dr. Frith did meet with Dr. Rudolf Stejskal, Searle Pathologist, at the conclusion of this review and discussed some of his findings with him.

The first phase of Dr. Frith's review consisted of the examination of the tissues of 25 of the surviving control females and 11 of the non-surviving control females for a total of 36 animals. All of the slides were examined for each animal and the results

were compared to the microscopic reports provided by Searle Laboratories. The inconsistencies (findings that differed from those reported by Searle) are listed below:

In most cases the inconsistencies represent findings that were not diagnosed or reported by Searle. Copies of Searle's microscopic pathology reports for each of the animals listed below are attached as exhibit #60.

Female Rat No. F13CF (Path. No. 95617)
Small Intestine - Diverticulum with mucosal necrosis and cellular inflammatory infiltrate.

Female Rat No. F15CF (Path No. 95618)
Pancreas - Focal hyperplasia.

Female Rat No. F16CF (Path. No. 95619)
Heart - Focal Fibrosis.
Kidney - Mild chronic nephritis.

Female Rat No. H10CF (Path. 95624)
Ovary - Neoplasm - probably granulosa cell tumor.

Female Rat No. H19CF (Path. No. 95626)
Kidney - Focal calcification.
Ovary - Neoplasm - probably granulosa cell tumor.

Female Rat No. H30CF (Path. No. 95628)
Kidney - Focal calcification.

Female Rat No. K25CF (Path No. 95630)
Kidney - Focal calcification.

Female Rat No. K29CF (Path No. 95631)
Heart - Focal fibrosis.
Kidney - Focal calcification.

Female Rat No. M4CF (Path No. 95632)
Liver - Focal hyperplasia.

Female Rat No. M10CF (Path. No 95634)
Kidney - Focal calcification.
Pituitary - Adenoma.
Ovary - Fibrosis and Pigmentation.

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Female Rat No. M15CF (Path No. 95635)
Pituitary - Adenoma.
Ovary - Cyst.

Female Rat No. B30CF (Path. No. 95801)
Kidney - Focal calcification.

Female Rat D29CF (Path. No. 95803)
Urinary Bladder (1) Chronic diffuse inflammation.
(2) Diffuse mild hyperplasia.

The second phase of the review consisted of the microscopic examination of all tissues from the high dose females - a total of 36 animals. The inconsistencies are listed below:

Female Rat No. B14HF (Path. No. 95657)
Eye was reported as not examined but eye was present and normal.

Female Rat No. F25HF (Path. No. 95823)
Urinary Bladder - Mild diffuse hyperplasia.

Female Rat No. H7HF (Path No. 95623)
Ovary - Neoplasm - probably granulosa cell tumor.

Female Rat No. H9HF (Path No. 95665)
Heart - Focal fibrosis.
Urinary Bladder - Mild focal hyperplasia.

Female Rat No. H15HF (Path No. 95666)
Lymph Node - The diagnosis of lymphoma, benign, was present on the Searle microscopic report. According to Dr. Frith, Lymphoma is generally not considered to be benign and he would diagnose lymphosarcoma.

Female Rat No. H18HF (Path No. 95667)
Pituitary - Adenoma.
Brain - Mild bilateral hydrocephalus.

Female Rat No. K18HF (Path. No. 95824)
Pituitary - Adenoma.

Female Rat No. K24HF (Path. No. 95671)
Mass noted grossly - nothing consistent with mass reported microscopically.

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Female Rat No. M2HF (Path. No. 95672)
Uterus - Chronic mild endometritis.

Female Rat No. M30HF (Path. No. 95343)
Kidney - Focal calcification.
Uterus - Chronic mild endometritis.

Female Rat No. M30HF (Path. No. 95675)
Pancreas - Focal hyperplasia.

The third phase of this review consisted of microscopic verification of all masses reported grossly at necropsy from all female animals not examined in phases 1 and 2 and included a total of 73 animals. The inconsistencies are listed below:

Female Rat No. D10LF (Path No. 92521)
Subcutaneous mass was diagnosed as an angiofibroma on Searle report. The lesion is more consistant with an angiosarcoma.

Female Rat No. K9MF (Path. No. 95707)
Uterus - Polyp.

Female Rat No. M1LF (Path. No. 95844)
Tissue mass seen grossly was reported as missing and not available for microscopic examination. The tissue was present and was a mammary fibroadenoma.

In summary, Dr. Frith reviewed:

1) All 36 high dose females (all slides) including 3 that had been excluded from the study due to autolysis.

2) 36 (one-half) of the control females (all slides) including 1 animal that had been excluded from the study due to autolysis.

3) Remaining 73 female animals with grossly observed masses. (sufficient slides were reviewed to substantiate the masses)

4) 5 additional animals selected by the investigators (A1HM, A9HM, A29HM, C2CM, C24HM).

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The slides reviewed in the first two categories above constituted 20% of the total animals on the study. Dr. Frith reviewed these slides blindly and then compared his findings with the Searle microscopic reports. According to Dr. Frith, his findings were in agreement with those of Searle, for the most part. In his opinion, some of the lesions that he reported as inconsistencies were small, and might be considered insignificant by some pathologists. Dr. Frith did feel, however, that the ovarian neoplasms (animals H10CF, H19CF, and H7HF) and caronic cystitis and diffuse hyperplasia (animal D29CF) should have been reported.

Dr. Frith also considered two other discrepancies to be significant. They were:

1) The reporting of a mass (by Searle) as missing which was actually present (M1LF).

2) The finding of a polyp of the uterus which was not diagnosed by Searle (K9MF)

The second of the above two discrepancies assumes even more significance in view of the following:

The Histopathologic Summary table (table 11) in Volume I of the submission to FDA lists the following incidence of Uterine Polyps on page 87:

Incidence of Uterine Polyps

Controls	Low	Medium	High
1 of 69 (1%)	1 of 34 (3%)	4 of 34 (12%)	6 of 33 (18%)

The finding of one additional uterine polyp by Dr. Frith (in animal K9MF) increases the incidence in the mid dose to 5 of 34 (15%).

On page 82 of Volume I of the submission to FDA, is the statement: "other sporadic findings included endometrial hyperplasia, polyp, cyst, congestion and squamous metaplasia." The term "sporadic findings" was used to characterize the incidence of uterine polyps, in spite of the fact that Searle had done a statistical analysis of these findings.

When this study was reviewed by the Bureau of Foods in 1975, the dose-related incidence of uterine polyps was noted. The appropriate slides were requested by FDA at that time and were reviewed by three groups of pathologists: 1.) The Division of Pathology, Bureau of Foods, 2.) Armed Forces Institute of Pathology, 3.) Massachusetts Institute of Technology. Copies of the reports submitted by the 3 groups and related correspondence were obtained and are attached as exhibits #43-45.

Dr. Rudolph Stejskal was responsible for the microscopic findings and accuracy on these findings in the submission to FDA. Only Dr. Stejskal's name appears on the submission. However, a Dr. Joseph H. Smith, M.D. also read slides for this study and his initials appear on some of the microscopic examination sheets. Dr. Frith questioned some of the terminology used in describing tissues. Dr. Stejskal stated that Dr. Smith had come directly to Searle from a hospital situation. Due to his human pathology background, his description of animal tissues was somewhat different than that used by veterinary pathologists.

Dr. Stejskal joined Searle in July of 1973, therefore, he had no input into the pathology protocol, since E-77/78 was initiated in November of 1971.

No microscopic worksheets or other "raw data" relating to microscopic pathology could be found for study E-77/78. We were told by Searle personnel that the original microscopic findings were dictated by the pathologists (Stejskal & Smith) onto belts, and then typed onto sheets which were placed in a binder. The belts were then discarded and apparently the bound microscopic pathology sheets were either discarded or lost, after the study report was written. Therefore our verification of the microscopic findings submitted to FDA was limited to a complete inventory of the slides and tissue blocks, and microscopic examination of a representative number of slides by Dr. Frith.

Our inventory of the slides and tissue blocks for each animal included a complete list on the tissues sectioned, the number of slides made from each tissue, and a complete count of the total number of slides and blocks for each animal. We also checked the identification numbers on every slide and tissue block. We examined a total of 7,872 slides and 7,360 tissue blocks. The average number of organs submitted for tissue processing was

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20 per animal. No errors in slide identification were noted, although in many cases the number of organs submitted for sectioning was less than specified in the protocol. A detailed discussion of this can be found under the heading, PROTOCOL.

In addition to the discrepancies noted by Dr. Frith, some other errors were noted in the submission to FDA. A mammary tumor found in rat F27CF was described as a papillary cystadenoma on the individual pathology sheet (page 105, Volume II of the submission to FDA) and as an adenocarcinoma on the summary table 12, page 96, Volume I of the submission to FDA.

Page 92, Volume I of the submission to FDA (a summary table) reports that animal J23CM was found dead after 754 days on study, while the individual pathology sheet for this animal (page 56, Volume II of the submission to FDA) reported that the animal was found dead after 620 days on study. The correct figure is 620 days, since J23CM was placed on the study on 11/17/72 and was found dead on 7/29/73.

In several instances the histopathology technician made notes at the bottom of the gross pathology sheet to indicate that certain organs were not present in the bottle of fixative. (and were therefore not available for sectioning). Yet in three of these instances (animals A4CM, K23CF, and J3CM) a diagnosis appears in the submission to FDA.

CHARTS, DIAGRAMS AND TABLES

It was necessary to construct a number of charts, diagrams and tables to facilitate our review of the data. For example we constructed a chart, by housing group, showing the identification and complete pathology history of each of the 360 animals. We also rearranged this chart into dosage groups, a copy of which is attached as exhibit #35.

To compare survival data it was necessary to construct a survival table. This also involved devising a calendar to show days and weeks on study for each housing group, taking into account the starting dates for each group. This also included tables showing the numbers of days and weeks animals were on study and a table comparing the survival data from various sources.

We constructed a chart showing diet calculations (gm./kg) and total amounts of DKP used (gm./batch). This is attached as exhibit #30.

Three tables were constructed which summarize the FDA statistical analysis of body/feeder weight data. They are attached as exhibits #39-41.

All of the charts, diagrams and tables that we constructed are attached to the report as exhibits and are referred to in various sections of the report.

EXHIBITS

#1. G.D. Searle & Company Annual Report for 1976.
#2. Organizational Chart of Pharmaceutical/Consumer Products Group.
#3. Organizational Chart of World Wide Pharmaceutical R&D Group.
#4. Organizational Chart of Preclinical R&D Group.
#5. Organizational Chart of Product Safety Assessment Group.
#6. Copies of Computer-Generated Randomization Tables used by Searle to assign the Dose & Housing Groups.
#7. Diagram showing Typical Housing Group of 30 animals, containing a random distribution of control and treated animals.
#8. Diagram showing arrangement of food cups on cart, used in feeding the animals.
#9. Copy of "Glossary of Terms for Aspartame and its Diketopiperazine" and "Analytical Data and Specifictaions of Food Grade Aspartame".
#10. Copy of shipping labels for rats received from

#11. Copy of protocol with amendments for Study P.T. 988S73 (E-77/78).
#12. Copies of CV's for prinicipal persons involved in study E-77/78.
#13. Copies of Batch Records for the manufacture of DKP, lots 1R through 5R.
#14. Copies of pages from Searle chemist Jack Drogt's notebook, concerning the manufacture of DKP.
#15. Copies of Analytical Reports for DKP, lots 1R through 7R.
#16. Copy of Searle memorandum dated 12/4/69, concerning DKP Specifications.
#17. Copy of DKP Specification Sheet (not dated) entitled "Tentative Specifications for SC-19192".

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#18. Copy of DKP Specification Sheet entitled "Specifications for SC-19192, Specification #C40606C".
#19. Copies of pages 75-84 & 285 of lab notebook #AR-39, concerning assay of DKP, lots 1R, 2R & 3R.
#20. Copies of pages 60-63 of lab notebook VSH-I, and page 269 of lab notebook #AR-23, pertaining to analysis of DKP lot 4R.
#21. Copies of pages 250, 251 and 257 of lab notebook #AR-57, and pages 44-49 of lab notebook #AR-68, pertaining to analysis of DKP lot 5R.
#22. Copies of pages 83-86 of lab notebook #AR-77, concerning analysis of DKP lot 6R.
#23. Copy of page 31 of lab notebook #AR-93, concerning analysis of DKP lot 7R.
#24. Copy of protocol for DKP stability study, dated 1/13/72.
#25. Copies of pages 51-56 of laboratory notebook #AR-49, assigned to C. Saul. These pages describe a preliminary TLC Test for recovery of DKP from the diet mixture.
#26. Copies of pages 53-59, 67-72, 88-89, 106-107, 144-145, 156-157, and 284-285 of laboratory notebook #AR-51, assigned to Barbara Bickford. These pages refer to the assay procedure and methods for the DKP Stability Study.
#27. Copies of Analytical Reports for DKP Stability Study.
#28. Copies of DKP Compound Inventory Cards.
#29. Two photographs showing a non-homogeneous sample of DKP diet mixture.
#30. Chart showing diet calculations (gm./kg.) and total amounts of DKP used (gm./batch).
#31. Two memos dated 7/14/77 from Thomas F.X. Collins concerning interview with Ray Schroeder.
#32. Memo dated 7/19/77 from Thomas F.X. Collins describing the 7/18/77 interview with Ray Schroeder.
#33. Memo of Telephone Conversation between Jerome Bressler and Attorney John H. Bickley Jr., dated July 25, 1977.
#34. Copies of records concerning calculation of diet concentrations, food concentration prediction records, dates of batch mixing, and calculation of mean food intake values.
#35. Charts organized by dose group, showing the identification and pathology history of each of the 360 animals on study.
#36. Memo dated April 5, 1976, from Dr. John H. Rust to Dr. R. McConnell.
#37. Searle memo dated September 30, 1974, by Dr. McConnell.

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#38. Memo dated August 29, 1974, from Dr. G.L. Schoenhard to Dr. K.S. Rao.
#39. Table 1 - Summary of Average Body Weights and Weight Gain (& change/week) from the FDA Statistical Analysis.
#40 Table 2 - Summary of Food Intake (g/day and g/kg./day) and dosage (mg./kg./day) from the FDA Statistical Analysis
#41. Table 3 - Summary of Food Efficiency (g. gained/100g. actual food consumed) calculated in the FDA Statistical Analysis.
#42. Computer printout of FDA Statistical Analysis of food intake and body weight data.
#43. Pathology report from Division of Pathology, Bureau of Foods, concerning uterine polyps, along with correspondence, and memo from Janet Springer.
#44. Pathology report from Armed Forces Institute of Pathology, concerning uterine polyps.
#45. Pathology report from Massachusetts Institute of Technology, concerning uterine polyps.
#46. Written account of interviews with Dr. Jean Taylor on 6/2/77, 6/3/77, and 6/7/77.
#47. Written account of interview with Judy Beauchamp on 6/2/77.
#48. Written account of interview with Barbara Bickford on 6/1/77.
#49. Written account of interview with Clifford J. Seul on 6/2/77.
#50. Written account of interview with Bartolome R. Tangonan on 6/1/77.
#51. Written account of interviews with Tony Martinez on 5/19/77, 6/3/77, 7/7/77, 7/20/77, and 8/2/77.
#52. Written account of interview with Ted Reichert on 5/24/77.
#53. Written account of interview with Barbara Bickford and Clif Seul on 6/2/77.
#54. Written account of interview with Judith Schmal on 6/2/77, and 6/7/77.
#55. List of animals bled at 104 and 114 weeks.
#56. Written account of interview with Alan Mitchell on 7/20/77.
#57. Written account of interview with Raymond G. Schroeder on 7/18/77.
#58. Injection records showing administration of penicillin, dates of administration, rat numbers, and units injected.
#59. Methodology for "Phenistix" determination of phenylketones in urine.

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#60. Dr. Frith's report of examination of slides for DKP Study (E-77/78).
#61. Key for animal identification card numbers used on the body/feeder weight teletype sheets.
#62. Chart correlating animal cage numbers with pathology numbers, arranged by dose group.
#63. Chronological list of pathology numbers and corresponding animal cage numbers.
#64. Organizational charts showing responsibility during the time that study E-77/78 was conducted.
#65. Volume entitled "tissue masses & deaths". Chronological list of all animals that died, during study, and dates that masses were first observed.
#66. Charts of days on study for each animal.
#67. Calendar for duration of study showing starting dates, days and weeks for each group.
#68. Survival table.
#69. Charts of Housing/Dosage Groups.
#70. "Observations for Drug Effects" records for housing groups A through F.
#71. "Observations for Drug Effects" records for housing groups G through M.
#72. Ophthalmoscopic records and copies of pathology sheets that have ophthalmoscopic findings.
#73. Life table analysis and statistics on body weight and food consumption data by Dennis Wilson, Div. of Mathematics, Bureau of Foods.
#74. Evaluation of feeding study on DKP, a conversion product of Aspartame, by Janet Springer/Ann Ducca, FDA Division on Mathematics.
#75. Volume A - teletype sheets for body and feeder weight data, housing groups.
Volume B - "
Volume C - "
#76. Copy of Searle Computer Program.
#77. Volume of protocols, organ weights, dosage, hematotogy, urinalysis, blood chemistry, and protein electrophoresis.
#78. Complete gross pathology sheets, males.
#79. Complete gross pathology sheets, females.
#80. Key to slide tissue identification numbers and abbreviations.
#81. Key to stain abbreviations.

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#82. Copies of submission appendix tables relating to hematology, clinical chemistry, urinalysis, and electrophoresis, along with check marks showing errors, and attached copies of raw data sheets documenting the errors.
#83. Copies of submission appendix tables for organ weights, with errors indicated, and copies of pathology sheets documenting the errors.
#84 Data sheets showing the phenylketones test erroneously labeled "phenylalanine".

John S. Arnold
Investigator

David M. Erspaner
Investigator

Dr. Jean Taylor
Toxicologist

Dr. Leonard Friedman
Biochemist

The following is the 20%, the investigation of the mouse studies. FDA did not want you to see them and they deleted them. It is hereby added to the back of the report.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

September 26, 1979

CHICAGO DISTRICT
1222—POST OFFICE BUILDING
433 WEST VAN BUREN STREET
CHICAGO, ILLINOIS 60607
TELEPHONE: 312 353-5863

John W. Olney, M. D.
Department of Psychiatry
Barnes and Renard Hospitals
4940 Audubon Avenue
St. Louis, Missouri 63110

Re: FOI # 79-21004
CHI- 1766

Dear Dr. Olney:

This is in response to your request of August 20, 1979 for records from the Food and Drug Administration pursuant to the Freedom of Information Act: Paragraph III of your letter re: G. D. Searle's product: Aspartame.

X We are enclosing the requested record(s) consisting of EIR dated 4/25 - 8/4/77 regarding study of E 77/78 and EIR dated 5/2 - 7/8/77 regarding study E-5 and E89.

X As you will note, minor deletions of material have been made in the records furnished to you. In the judgment of the Food and Drug Administration, the information deleted does not fall within the scope of your request and, in any case, is not required to be disclosed under the Freedom of Information Act. If, however, you do desire to review the deleted material, please make an additional request. If the agency should then deny you this information, you would have the right to appeal such denial to the Department of Health, Education, and Welfare. Any letter of denial will tell you how to make this appeal.

____ No deletions were made from this material.

____ The requested record(s) will be sent at a later date.

X Other: I apologize for the quality of the 5/2 - 7/77 EIR. The problems were with our original copy. Please contact me at 312-353-5863 if you have problems.

X We are assessing the following charges:

125 pages	@ $ .10 =	$12.50
2 hours search time -1/2 hour	@ 3.00 =	4.50
Total		$17.00

An invoice is attached.

____ The charges will be aggregated to your bill.

____ There will be no charge for furnishing record(s).

Sincerely,

George F. Bailey

George F. Bailey
Freedom of Information Officer

Enclosure: a/s
GFB/ed

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SUMMARY OF FINDINGS

We made a detailed inspection of the raw data versus the final report on two teratology studies on SC 18862 (aspartame). These studies, numbered E-5 (PT851S70) and E-89 (PT1218S75) were selected for our inspectional coverage by headquarters personnel of the Bureau of Foods. Study number E-5, "SC-18862: Evaluation of Embryotoxic and Teratogenic Potential in the Rat" had not been previously inspected by FDA personnel at Searle Laboratories. Study number E-89 was included as one of five teratology/reproduction studies that were covered by an FDA inspection team during the period of December 1 through 19, 1975.

Our inspection of Study E-5 included the following findings:

1. The individual doing the examinations of the visceral and skeleton specimens was aware of the dose levels. The examinations were not done blind.

2. There are no individual fetus records for the skeletal examinations. The skeletal examination data is listed only by litter under the dam number. The skeletal examination records are not dated.

3. There are no examination sheets that specify the abnormalities that are included in their examination of visceral sections. Their visceral examination records indicate only "O.K." if no abnormalities were found. The visceral examination sheets do not list the respective fetus identification numbers for about 10% of the 329 fetus visceral specimens. These incompletely identified fetus specimens are identified on the examination sheet with only the dam number and fetus sex.

4. According to the visceral examination records, a total of 329 visceral examinations might have been done on two days. We were unable to examine any visceral sections from study E-5 because they had been discarded.

5. There were no signatures or initials to identify the individuals who did the work on the skeletal, visceral, and laporotomy examination sheets.

6. There was no identification on the body of the vials holding the skeleton specimens; the respective fetus number was on the vial cap (See exhibit 39, photo 1).

7. There was no record to verify the source and age of the male rats.

8. There were no specifications or assay records on the basal diet.

9. There were no batch records for the mixing of the powdered SC 18862 (aspartame) with the meal form of Rockland diet (Teklad Inc.) Mrs. D. Helms, Research Assistant, could not remember the details of mixing - such as the total mixing time or the order of adding the SC 18862 and the Rockland Diet to the mixer.

10. The treatment mixtures (two dose levels) were not assayed for potency, homogeneity or stability.

11. The examination of the fetal skeletons of 5 litters of each dose level by Dr. T. Collins revealed only a few differences from their original skeletal examination data as compared to the FDA submission. A few differences in the results are not unusual between 2 individuals when they are doing examinations. These findings are detailed in the body of the report.

Our inspection of study number E-89 included the following findings:

1. The individual who did the visceral and skeleton examination was aware of the dose level of the specimens that were being examined.

2. There are no examination sheets that specify the abnormalities that are included in Searle's examination of visceral sections.

3. The only identification of the skeleton specimens is on the caps of the vials with the respective fetus number and the PT number, 1218.

4. The records covered receipt of only 10 of the 36 male ~~rats~~ mice.

5. There were no signatures or initials to identify the individuals who did the work on the skeleton examination records.

6. There were no assay reports or specifications on the basal diet.

7. There were no batch records for the mixing of the aspartame with the [redacted] chow. The three treatment mixtures were not assayed for potency, homogeneity or stability.

8. Searle did not include any abnormal findings of visceral examination in the report that was submitted to FDA. The raw

data included major malformations of a segmented uterus in a low dose fetus 20407 and a cleft palate in a medium dose fetus 32012 neither of which was included in the FDA submission. Dr. Vondruska was shown this data and said this omission was an oversight (see Vondruska's interview). Dr. Collins examined visceral sections that included verification of the aforementioned findings. Dr. Collins also noted a slight hydrocephalus of fetus 20407, low dose, that was not in the raw data or the FDA submission. This was confirmed by Dr. J. Noverosky the Searle Teratologist. (see exhibit 39, photo 2) Dr. Collins disagreed with Searle's classification of "renal pelvic cavitation of the kidney not enlarged" of the fetus 41101 as an artifact and not a malformation. (see exhibit 39, photo 4) Dr. Collins does not agree that this is an artifact and he is of the opinion that it is due to the blockage of the urinary tract.

Dr. Vondruska stated that in retrospect "artifact" was probably a poor word to use. He said that Coll might have sectioned the kidneys at an incorrect angle, thereby, giving the appearance of an enlarged renal pelvis. (see Vondruska interview)

9. It would appear that the visceral sections were cut too thick. There would be a possibility that some visceral abnormalities would be missed.

10. It was noted in the FDA submission that there was a significantly greater number of fetuses in the medium dose level with poorly ossified supraoccipital bones, when compared to the control group. Because of this finding, the supraoccipital bones of the fetuses in the high dose level were examined. Dr. Collins scanned the supraoccipital bone for poor ossification in each of the skeletal fetuses of the control and high dosage groups. His examination of the supraoccipital bone revealed the following percentage differences from the FDA submission.

Supraoccipital Bone Poorly Ossified	Control Fetuses	High Dose Fetuses
FDA Submission	3%	6%
Examination by Dr. T. Collins	4.46%	8.47%

11. C. Kirby, Reasearch Technician whose duties included the visceral and skeletal fetal examinations and laparotomies for study E-89 completed about three years of college. She started employment with Searle Laboratories in August of 1974 and performed visceral and skeletal exams on E-89 in May and June of 1975. This was the only study where she performed the visceral exams. She stated that her on-the-job training consisted of a total of about 3 months.

12. There are no dates of examination on the skeleton tables (exhibit 30). On the back of the laparotomy sheets, the major skeletal variations are listed. Most of the skeletal examinations are dated 5/19/75 and 6/4/75. It would be impossible for one individual to do a complete skeletal examination of over 500 fetuses in 2 days. It is unclear over what period of time these fetuses were read.

PURPOSE OF INVESTIGATION

Assignment memo dated May 16, 1977 from Donald Healton, Acting Executive Director of Regional Operations, confirmed an earlier oral assignment to Chicago District for a directed inspection of certain non-clinical studies submitted to FDA in support of a food additive petition for the sweetener, Aspartame.

The investigating began on 4/25/77 (see EIR E 77/78) and encompassed the authentication of all data, both raw and summary, relating to the studies jointly chosen for review by the Bureau of Foods and EDRO. Two studies actually done at G.D. Searle were selected for initial coverage, and a decision to expand the investigation to a third study was made at a later date.

We began our investigation of E-5 (PT-851S70) Evaluations of Embryotoxic and Teratogenic Potential in the rat, using SC18862 (Aspartame), on May 2, 1977.

On May 11, 1977, after clearance from the Bureau of Foods, we initiated the investigation of E-89 (PT-1218S75) an Evaluation of Embryotoxic and Teratogenic Potential in the mouse, using SC-18862 (Aspartame), see assignment attached.

This report is concerned with the above two studies. The report involving E-77/78 will be reported separately.

REFUSALS

Attached as exhibit 40 is a memorandum dated June 29, 1977 from Mr. Roger Thies, Attorney refusing our request for an additional interview of Ms. Gail Kirby, a technician who worked on E-89 (PT-1218S75), Evaluation of Embryotoxic and Teratogenic Potential in the mouse (aspartame).

We were concerned with the dates shown on the back of the laparotomy sheets, "6/14/75" and "5/19/75." Dr. Collins is of the opinion that it would be extremely difficult to completely examins 300 skeletons in two days, if these dates, so indicate. In our interview with Mr. Schroeder, a former employee, he told us that he was able to examine thirty skeletons in a day. (see Schoeder interview attached) In our interview with Dr. Vondruska, he could not not explain the dates shown on the back of the laparotomy sheets. He told Dr. Collins that he would have to ask Ms. Gail Kirby.

Our failure to interview Ms. Gail Kirby leaves the question of the dates unresolved. G.D. Searle's refusal to allow us to conduct a telephone interview is given in the menorandum from Mr. Thies (see exhibit 40). We do not consider his reasons for refusal as valid.

PERSONS INTERVIEWED

Investigators Carl E. Lorentzson and Johnny F. Salas presented their credentials and issued a Notice of Inspection on May 2, 1977 to Richard E. Viktora, Attorney. Dr. Thomas F.X. Collins issued a Notice of Inspection on May 4, 1977 to Dr. William M. Merino, Director of Regulatory Affairs. Dr. Collins was at Searle Laboratories on May 4-5, 23-27, June 6-7, and July 7 and 8, 1977. Investigators Carl E. Lorentzson and/or Johnny F. Salas were both present on each date of inspection with the exception of July 7 and 8, 1977 Investigator J. Salas was present at Searle Laboratories for the inspection of studies E-89 and E-5 on May 17, 1977, when Investigator C. Lorentzson was not at Searle Laboratories. An attorney and/or a Ph.D. from one of the research units of Searle Laboratories was present whenever we reviewed records, inspected the facilities, examined

fetal skeletons or interviewed personnel. These individuals were:

Dr. Robert Bost - Director of Food Products, Regulatory Affairs
Dr. George Clay - CNS Group Leader
Richard Viktora - Attorney
Roger Thies - Attorney
Dr. J. Nevedroske - Group Leader of Toxicology
Dr. Fred A. Radzialowski - Section Leader of Cardio-vascular Pharmacology
Dr. R. Jenkins - Director of Product Affairs
Dr. Richard L. Aspinall - Group Leader of Immunology & Inflammatory Diseases

We interviewed Research Assistant Mrs. D. Helms at Searle Laboratories regarding her duties on study E-5.

We made arrangements to interview Raymond Schroeder, a former employee whose title at the time was Senior Research Assistant, and whose principal duties were on study E-5 and relatively limited duties on study E-99. This interview was conducted in New Jersey because Raymond Schroeder is now residing in Somerville, N.J.

We interviewed the following individuals regarding their duties on study E-99:

1. Gail Kirby - Research Technician
2. Jeanne Thompson - Research Technician
3. Dr. J.P. Vondruska - Senior Investigator
4. Alan Mitchell - Teratologist

Richard Viktora provided us with the date that Raymond D. Schroeder left this firm, namely May 2, 1975. However, Mr. Viktora said that he would not furnish a copy of a record to substantiate this termination date because it would be a violation of the Equal Employment Opportunity Regulations. We were allowed to review and make notes from the following records. However, Roger Thies, Attorney, did not allow photocopies because he did not consider these records to be primary data on study E-5, namely:

1. A preliminary draft of the summary and conclusions for the final report on "AY 851570" (Searle Doc #114652)

2. A list of the studies which either have been completed or were in progress with aspartame to determine the relative toxicity of aspartame and Diketopiperazine in several species of animals. (Searle Doc #127235B)

3. A "galley copy" of the report that was submitted to FDA

4. An inventory list of the teratology specimens that were stored in a basement storage area. The record included a listing of the fetal skeleton preparation from the rat, in study E-5 (PT-851S70) in box numbers T-043A, T015.

SCOPE OF OUR INSPECTION

We requested all of the records pertaining to study E-5 on the first day of our inspection, May 2, 1977. It was brought to our attention by Jerome Bressler, FDA inspection team leader, that the data pertaining to this teratology study had been previously placed under FDA seal. We then visited their R&D central file room to locate these records. We determined that the data including primary records pertaining to their teratology studies on SC 18862 (aspartame) was stored under FDA seal in two file drawers. We initially attempted to remove the data from these file drawers that pertained only to study E-5. In order to facilitate our detailed examination of these records on teratology studies, we then removed the records on all of the teratology studies in their two file drawers to a room on the first floor of "J" building. Whenever we did not personally guard these records, we maintained the data on these teratology studies in a locked metal cabinet under FDA seal. We obtained almost all of the records for our E-5 study from their central file room. We subsequently requested additional records pertaining to the study E-5 such as the lab testing of the component, SC 18862; invoice for purchase of female rats; curriculum vitae and chain of responsibility. We made photocopies of essentially all primary data and other records pertaining to study E-5. Exhibit numbers 1 through 13, 38 and 3 photos in Exhibit 39 pertain to study E-5.

We made a detailed review of all raw data against the report that was submitted to FDA. This review included fetal and maternal body weights, maternal food consumption, crown rump measurements, number of corpora lutea, number of live and dead fetuses and examination records on visceral and skeletal fetal specimens. Dr. T. Collins examined skeletal specimens from study E-5, and skeletal and visceral specimens from study E-89.

After we completed the majority of our inspectional work at Searle Laboratories on study E-5, we received authorization from personnel

of the Bureau of Foods on May 11, 1977 to institute an inspection of an additional teratology study, E-89 (FT-1218975) entitled - "SC-18862 - An Evlauation of the Embryotoxic and Teratogenic Potential in the Mouse". We made copies of all primary data and other records pertaining to study E-89. Exhibit numbers 16 through 38 and photo numbers 2, 3, and 4 of Exhibit 39 pertain to study E-89. We made a comprehensive review of all raw data with one minor exception. We estimate that we checked more than one third of the food consumption primary data for accuracy. The previous inspection of December 1-19, 1975 included study E-89 and stated in part that maternal food consumption was transferred without error from the raw data. The FDA submission on study E-89 states in part that the pregnant animals actually consumed dose levels for the low, medium, and high dose groups respectively which are approximately 40% more than the originally intended doses of 1.0, 2.0 and 4.0 g/Kg.

PERSONNEL ON THE E-5 STUDY

Individual	Title & Background	Duties
Mrs. Donna Helms	Research Assistant Her educational background includes B.S. Univ. of Wisconsin with a major in Zoology in 1966. She started work for Searle Laboratories in 1969 and is currently employed by the firm.	Donna Helms stated that her duties included: weighing of the animals; setting up the study; food consumption data; transfer of data from cage cards to laparotomy sheets; and performing hysterotomies.
Raymond F. Schroeder	Senior Research Assistant in Teratology. His education includes a M.S. in Zoology from the Univ. of Illinois in 1967. He was employed by Searle Laboratories from Dec., 1967 to May 2, 1975.	According to Donna Helms, the duties of Ray Schroeder included external observation of the fetuses; supervision of the laparotomy; and performance of the visceral sections and skeletal examinations.

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Margaret S. Faber (Hoppenrath)	Bio Research Technician	Donna Helms stated that Margaret Faber might have done some of the crown rump measurements. Donna Helms was unable to recall any other work that was done by Margaret Faber on study #2-5. Raymond Schroeder informed us during his interview that the duties of Margaret Faber (Hoppenrath) included: killing of animals, mixing of the diet, crown-rump measurements, weighing of fetuses, staining of skeletons, and cutting the visceral sections.

Copies of Curriculum vitae for key personnel and a listing of the responsible individuals of Searle Laboratories during the years 1969 and 1970 are attached as exhibits numbered 1 and 2. Study number 2-5 was conducted during the first half of 1970. The Director of Biology of Searle Laboratories during this time period was V.A. Drill. The authors of the report are R.E. Schroeder and R.C. McConnell, Dept. of Pathology-Toxicology, Division of Biological Research.

Study 2-5 (PT 851570)

SC-16662: Evaluation of the Embryotoxic and Teratogenic Potential in the Rat

Date study initiated: Jan. 20, 1970

Dates of performing laparotomies: Feb. 9 through Feb. 19, 1970

Date study was received by Bureau of Foods: August 7, 1972

Animals:

Species and Strain - Albino rat, Charles River caesarian derived virgin females and proven males

Number and Sex - 90 females, 30 males - there were no records to indicate source and age of male rats. We verified that the females were approximately 10t days old at time of mating - Invoice (Exhibit 4) indicates date of receipt: 12/30/69.

Experimental design:

Ninety females were distributed into the following three groups. Mrs. D. Helms said that she used a randomization method that involved drawing animal numbers from pieces of paper in a hat. She didn't remember if the first number drawn was assigned to a control group.

Dosage Group	No of animals	Dose Level - mg/kg
Control	30	0
Low	30	2000
High	30	4000

The respective identification number of each of the rats was punch marked on their ears.

Donna Helms could not state definitely whether the animals from each dose group had a unique color marking on their tail. Three females, one from each of three dosage groups were housed together in a breeding cage. At 4:30 p.m. one male was placed into each cage; he was removed at 8:30 a.m. the following morning. At that time females were examined for a copulatory vaginal plug and/or spermatozoa in the vaginal smear. Observation of either of these signs indicated mating and was designated day 0 of pregnancy. Such females were removed from the breeding cage and housed individually. They put this rat in the next empty cage going from left to right. This procedure was continued until a minimum of 24 females from each group were mated. Copies of the cage identification cards are attached as Exhibit number 6. We were informed that any daily observations would be recorded on these cage cards. There are no records of abnormal observations on these cards.

Dietary administration of SC-18862 at the dose indicated (2.34% and 5.00% concentration respectively) began on day 6 of gestation and continued through day 15 of gestation, a 10 day period of treatment. The females were sacrificed on day 20 of gestation. The uterine horns were exposed and examined. The fetuses were removed, examined externally and preserved intact to be examined later for visceral irregularities (Wilson Technique) or skeleton anomalies (Alizarin Red S Skeletal staining technique).

Dose Group	Bred	Surviving	Pregnant
Control	27	27	26
Low	25	25	24
High	24	24	23

Donna Helms could not remember the exact animal room in which this experiment took place. However, she showed us an animal room that closely resembled the actual room that was used to house the animals for study E-5. This room had only one doorway that was used for both the entrance and exit. The room had equipment to control the temperature and adjust the number of hours of light and darkness.

A photocopy of their protocol is attached as Exhibit 3. Our review of this protocol reveals that it is essentially in conformance with their FDA submission on study E-5 (Exhibit 13).

Formulation of SC 18862

The SC 18862 was mixed with the basal diet in weight per weight concentrations of 2.34% and 5.00 respectively for the low and high dose groups.

The [redacted] Model, V-1401 mixer that was used during 1976 in the research facility of Searle Laboratories in Skokie, Illinois was subsequently moved to another division of the G.D. Searle Company. This mixer was then returned to Searle Laboratories in Skokie, Ill. where it is currently being used in their Pharmaceutical Development area. We inspected this mixer (about 5 feet high) and noted that it was currently being used with a mixing bowl that would hold approximately 20 kilos of a treatment mixture. The treatment

mixture batch sizes for study E-5 were 3 kilos or smaller. It was brought to our attention that this mixer was formerly equipped with a smaller mixing bowl and a smaller anchor shaped mixing blade when it was used to mix treatment mixtures of aspartame in 1976. During our interview with R. Schroeder, he described a smaller mixer (about 2 feet high) with a 10-gallon capacity mixing bowl.

Searle did not maintain batch records for the mixing of powdered [redacted] with the meal form of Rockland Diet ([redacted]). There were no specifications or assay records on the Rockland Diet. We were informed that the manufacturer of the Basal Diet is out of business. Lot number 74020 of SC-18662, a white powder, obtained from the manufacturer [redacted] was incorporated in the treatment mixtures. This material was submitted to lab testing that included: identity tests; pH in water; melting range; specific rotation; total nitrogen content; loss on drying; heavy metals and thin layer chromatography. Copies of their records regarding lab testing of this lot of SC-18662 are attached as Exhibit 5.

The treatment mixtures (two dose levels) were not assayed for potency, homogeneity or stability. The treatment mixtures were mixed in the [redacted] mixer in the "Diet Kitchen" by Raymond Schroeder, Senior Research Assistant in Teratology or Mrs. Donna Helms, Research Assistant. Mrs. D. Helms could not remember the details of mixing such as the order of adding the SC-18662 and the Rockland Diet to the Hobart mixer. Reserve samples of the treatment mixtures were not maintained. Additional details regarding the treatment mixtures are included under a subsequent heading of "Interview of Raymond Schroeder."

Food Consumption

Copies of the food consumption records are attached as Exhibit 7. A quantity of food consumption that is underlined on these records indicates that a weighed quantity of spillage has been subtracted. Donna Helms said that food consumption was always measured first thing in the morning. Donna explained that in an attempt to account for food spillage she separated the food from the excreta on the tray beneath the respective animal cage. Donna Helms said that she covered the feed jars with a V-type mesh screen for the rats that were considered "chronic spillers". She said that the feed was transferred to smaller size jars during the course of the study in order to make it easier for the pregnant rats to reach their food. Our calculation of their food consumption records indicated that their statement regarding food consumption on

page 6 of the FDA submission is essentially correct. The amount of SC-18962 actually consumed closely approximated the planned dosages of 2000 and 4000 mg/Kg. On the basis of mean body weights on days 6 and 11 of gestation and mean food consumption from gestation days 6-15, the actual daily doses consumed by the low and high dose groups were 1,955 and 4,094 mg/Kg body weight respectively. Our calculations of the food consumption data revealed results that are within 1% of these reported average daily doses.

Hysterotomy Data

Donna Helms said that their hysterotomies were usually done in the morning. Their original records do not indicate observations of any lesions of the ovaries or uterus in any of the animals at sacrifice. The series of numbered hysterotomy sheets includes missing consecutive number hysterotomy sheets for animals that never mated. Our comparison of their original hysterotomy data (Exhibits 8, 9, & 10) and the tables numbered 1, 2, 3, & 4 in the FDA submission revealed only a few discrepancies. These hysterotomy tables included data i.e.: number of live and dead fetuses; sex of fetuses; number of resorptions; average fetal weight; and average crown rump measurements.

We noted the following discrepancies:

1. Table 2 of the FDA submission indicates that the average fetal weight for animal 29 of the control group is 4.0 grams; the average fetal weight for this animal is actually 3.9.

2. Original hysterotomy records indicate that there was one resorption on the "left" side for animal number 11 of the control group; table 2 of the FDA submission does not list this resorption on the left side. The FDA submission correctly lists the two resorptions that are marked on the right side of animal number 11 on their laparotomy sheet. Mr. R. Schroeder acknowledged these errors. (see R. Schroeder interview)

3. We noted the listing of one resorption for animal 72 on the laparotomy sheet; this resorption is not listed in the FDA submission. Mr. R. Schroeder acknowledged this omission and said it might have been a typographical error.

Skeletal Examinations - E-3

There are no individual fetus records for the skeletal examinations. The skeletal examination data is listed only by litter under the respective "Dam number". The skeleton examination records were not dated and did not bear any signatures or initials. Mr. E. Schroeder was shown these records and stated that they should have been dated. He said that it took a great deal of time to complete the skeletal readings. He also stated that it took him 5-6 minutes to do a complete skeletal examination of one fetus. (see interview with Mr. Schroeder) We compared the original skeletal examination records (Exhibit 11) with the report that was submitted to FDA (Exhibits 1-13). Dr. T. Collins also examined skeletal specimens of 5 litters of each dose level.

We noted the following:

1. The original skeletal examination records indicate a finding of "Hypoplasia of the Maxilla" in one fetus of Dam 57 and one fetus of Dam 58; (see exhibit 39, photo 6), this finding is not in the FDA submission. Mr. E. Schroeder acknowledged these errors.

2. The original skeletal examination records list a total of 166 (83%) fetal skeletons with unossified cervical centrum in the control group. The original records do not indicate how many of the cervical vertebrae had less than 3 ossified centra. The FDA submission indicates a total of 93 control fetuses had unossified cervical centrum with less than 3 centra ossified. It is probable that an error was made in transcribing the percentage of 83 instead of the total of 166 fetal skeletons with unossified cervica centrum to the FDA submission.

3. The original skeletal exam records indicate 3% upper and 1% lower incisors absent for the control group, 4% upper and 4% lower incisors absent for the low dose group and 5% upper incisors absent for the high dose. These are not mentioned in the FDA submission.

4. The original skeletal exam records indicate one sternum ossification center split for the control dose group; this sternum ossification split is not listed in the FDA submission.

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5. The rudimentary structures are small projections from the first lumbar vertebrae. These are small 14th ribs. Most animals with these structures are graded twice. They are counted as having 13 pairs of ribs as well as rudimentary structures.

6. Dr. T. Collins' examination of fetal skeletal specimens of 5 litters of each dose level revealed only a few differences from what was contained in the raw data that would alter the conclusion of the study.

7. Dr. Collins stated in effect that it would have been a better procedure to grade individual bones instead of closure grading for skeletal examination of the skull. (see Exhibit 11)

8. We made a physical inventory of the skeletal specimens. We compared this inventory against the skeletal fetal specimens that are designated on the laparotomy records (Exhibit 8, 9, & 10) as "A" for fetuses that were supposed to be initially preserved in 95% alcohol prior to staining, evisceration, clearing with aqueous potassium hydroxide, staining with Alizarin Red and storage of the skeletal preparation in glycerin. This inventory revealed that a total of 15 skeletal fetuses from the high dose group were missing. We were unable to obtain a definite reason as to why the following fetal skeletons were not in inventory: 6902, 6405, 8612, 8908, 8909, 8911, 8913, 9002, 9003, 9005, 9006, 9008, 9009, 9011, and 9013. Dr. J. Noveroske, Group Leader in Toxicology speculated that the four skeletal specimens from litter number 89 and the skeletal specimens from litter number 90 might be in a separate carton that was inadvertently misplaced.

9. Dr. T. Collins found mistakes in examining the skeletal specimens of all dose levels. As an example Dr. T. Collins noted a poorly ossified ischium for a fetus of Dam 58, this finding is not in the FDA submission. (see exhibit 39, photo 5) These mistakes appear to be equally distributed between the dose levels. Searle's examination of the skeletal specimens corresponds essentially with the FDA submission.

10. Raymond Schroeder, the individual who did skeleton examinations was aware of the dose levels of the fetal skeleton specimens. There is no identification on the body of the vials holding the skeletal specimens; the fetus number was marked on the vial cap. This method of identifying fetal skeleton specimens in vials could cause a mix up. Photo number 1 of Exhibit 39 illustrates their method of identification of the skeletal specimen on the cap of the vials.

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Control Group

Number of Litters - 26
Number of Fetal Skeletons - 201
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 34
Fetus Numbers Examined:

602	802	1402	2402	2702
603	803	1403	2403	2703
605	805	1405	2405	2705
606	806	1406	2406	2706
608	808	1408	2408	2708
609	809	1410	2409	2709
611	811			
	812			
	814			

Low Dosage Group

Number of Litters - 24
Number of Fetal Skeletons - 187
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 38
Fetus Numbers Examined:

3102	3902	4602	5302	5802
3103	3903	4603	5303	5803
3105	3905	4605	5305	5805
3106	3906	4606	5306	5806
3108	3908	4608	5308	5808
3109	3911	4609	5309	5809
3111	3913	4611	5311	5811
		4612		
		4614		
		4615		

High Dosage Group

Number of Litters - 23
Number of Fetal Skeletons - 187
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 36
Fetus Numbers Examined:

6102	7602	8002	8402	8702
6103	7603	8003	8403	8703
6105	7605	8005	8405	8705
6106	7606	8006	8406	8706
6108	7608	8008	8408	8708
6109	7609	8009	8409	
6111	7611	8011	8411	
		8012	8412	
			8416	

Visceral Examinations - Study F-5

Approximately one-third of the fetuses were fixed in Bouin's solution for subsequent examination by the free hand sectioning technique of Wilson. Tissue slices were examined under a dissecting microscope. The report submitted to FDA indicates that all tissue slices from treated fetuses and from control fetuses with anomalies were transferred to polyethylene bags for temporary storage. These specimens were discarded prior to our inspection and therefore we were unable to make any examinations of their visceral sections. There were no initials to identify the individual who did the work on the visceral examination sheets (Exhibit 12). The visceral examination records do not list the respective fetus identification numbers for about 10% of the 329 fetal visceral specimens. These incompletely identified fetus specimens are identified on the visceral examination records with only the dam number and fetus sex. As an example, a fetus of Dam number 40 would be listed as 40X female. The visceral examination sheet indicates only "O.K." if no abnormalities are found in the respective visceral section. There is no examination sheet that specifies what abnormalities they are particularly looking for in the visceral sections. The individual doing the examinations was aware of dose levels of the visceral specimens.

According to the visceral examination records, see exhibit 12, a total of 329 visceral examinations were done on two days, namely Feb. 27, 1970 and March 5, 1970. Mr. Schroeder said that he did visceral sections on approximately 30 fetuses per day.

The raw data and the report submitted to FDA specifies the finding of only three anomalies. Hydrocephalus was observed in one low dose and in one high dose fetus. Hydronephrosis and hydroureter were observed in one control fetus. We noted that the original visceral examination records also specified the finding of blood in the pericardial cavity of a visceral section of fetus number 4601 and the marking, "O.K.". This finding of blood in the pericardial cavity was not in the FDA submission. There were no other specific findings listed on the rat visceral examination sheets. The results of the remaining respective fetal visceral examinations were listed simply as "O.K.". It may be interesting to note that there have been teratology studies conducted in the rat by FDA laboratories where the findings in the visceral sections are reported for at least 10% of the fetuses.

COMPARISON OF THE LAPOROTOMY AND VISCERAL SHEETS

We uncovered at least 35 discrepancies when we compared listing of fetuses on the visceral and laporotomy examination record sheets (Exhibit numbers 8, 9, 10, and 12). Twenty-one of these discrepancies consist of listing a different sex for the respective fetus on the laparotomy and visceral examination sheets. The remainder of these 35 discrepancies include a listing of the alcohol fixative, (a) skeletal on the laporotomy sheets for fetuses that are listed on the visceral examination sheets or a listing of Bouin's fixative, (b) visceral on the laparotomy sheets for fetuses that are not listed on the visceral sheets. The following tabulation illustrates these discrepancies.

Visc. Exam Sheet	Lap. Exam Sheet	Comment
Not listed	4413 (B) Female	
4412 Female	4412 (A) Female	4412 is not in skeletal inventory
6104 Male	6104 (B) Female	
6110 Female	6110 (B) Male	
5801 Female	5801 (B) Male	
5810 Male	5810 (B) Female	
2110 Male	2110 (B) Female	
2113 Male	2113 (A) Male	2113 is not in skeletal inventory
1001 Female	1001 (B) Male	
1013 Male	1013 (B) Female	
7710 Female	7710 (B) Male	
7713 Male	7713 (B) Female	
Not listed	3012 (B) Female	3012 is not in skeletal inventory
3013 Male	3013 (A) Male	
2701 Male	2701 (B) Female	
2704 Female	2704 (B) Male	
804 Male	804 (B) Female	
813 Female	813 (B) Male	
2910 Male	2910 (B) Female	
Not listed	7212 (B) Female	
7217 Female	Not listed	DAM 72 had only 13 fetuses, it might refer to fetus #7212
3201 Male	3201 (B) Female	
6401 Male	6401 (B) Female	
6413 Female	6413 (B) Male	
3304 Male	3304 (B) Female	
3310 Female	3310 (B) Male	
1307 Female	1307 (B) Male	
1312 Female	1312 (A) Female	

C 6% L 6% H 4%

A total of 6 fetuses are listed for Dam #13	A total of 4 fetuses are listed in Bouin's (viaceral)	
Not listed	5504 (B) Male	5504 is not in skeletal inventory
5506 Female	Not Listed	Dam #55 had only 5 fetuses
A total of 6 fetuses are listed for Dam #39	A total of 5 fetuses are listed in Bouin's solution for Dam #39	
Not listed	9010 (B) Female	We were unable to locate any
Not listed	9012 (B) Female	of the skeletal fetuses for
9011 Female	9011 (A) Male	Dam #90 during our inventory of skeletal specimens

Study E-89

PT-1218575 - An evaluation of embryotoxic and teratogenic potential in the mouse - Aspartame (SC 18862) Seq. II

Before Dr. Collins, Bureau of Foods examined the visceral sections of this study it was brought to the attention of Searle's attorney, namely Mr. Roger Thies that some damage may occurr to these sections. The sections had been previously examined and it is a fact that these kinds of sections tend to come apart with age. These sections are approximately two years old.

Mr. Thies requested that official authorization in writing be given to Searle before Dr. Collins examined the visceral sections. Clearance and authorization was given by Mr. Richard Ronk, Director Division of Food and Color Additives. Dr. Collins was given authorization to examine the visceral sections of this study (E-89) in the company of a Searle teratologist. Dr. Collins agreed to inform Searle's teratologist the results of his readings, (see exhibit 41).

Date Initiated: Protocol Finalized - January 15, 1975
The first recorded body weight - February 27, 1975
The first recorded date of food consumption - February 27, 1975

Date Completed: Last body weight - April 14, 1975

Last food consumption - April 14, 1975

Final date on visceral exam worksheets - June 18, 1975; First date - May 28, 1975

Vondruska's notation on visceral examination of fetus 41101 female - 6/24/75

Dates Recorded on Reverse of Laporotomy Sheets For Skeletal Exams: The majority of the dates are either May 19, 1975 or June 4, 1975. Six fetuses of Dam 108 are listed with a skeleton exam date of 6/3/75.

Dates Recorded on Reverse of Laporotomy Sheets for Visceral Exams: May 28, 1975 and June 4,5,6,12,15,16,17 and 18

Date on Cover Sheet of Final Report Submitted to FDA: July, 1975

Animals Used: (\ Breeding Labs
Random bred albino mice, female CD-1 strain and
Random bred albino mice, males - proven breeders

36 females - Control
36 females - Low Dose
36 females - Medium Dose
36 females - High Dose

A copy of the purchase order for these females is attached as Exhibit #21.

Mating Procedure - natural mating; detection of copulatory plug designated as day 0 of gestation

	Concentration of SC-18862 in Diet (%)		Intended Daily Dose (Grams per Kilogram)
Dose Levels:	Low .75%	-	1 GPK
	Medium 1.5 %	-	2 GPK
	High 3.00%	-	4 GPK

Number of Pregnant Mice:

Control: 27
Low Dose: 25
Med. Dose: 27
High Dose: 21

We noted that the protocol specifies that body weights will be made on gestation days 1, 4, 6, 13, 15, and 18. The body weights in the FDA submission were recorded on gestation days 0, 1, 3, 6, 10, 13, 15, and 18.

Scope of the Investigation - E-89

We began a comprehensive review of Study E-89, PT 1218675 on 5/12/77, after the investigation of E-5, PT 851876 was essentially completed. This additional coverage was in accordance with authorization received from the Bureau of Foods.

We began our review by supervising the copying of all raw data stored under FDA seal at Searle Laboratories. These records include the following principal items:

1. Copy of protocol entitled Final Protocol For a Pre-clinical Safety Study of SC-18862 Path-Tox Proj. No. 1218575 (Exhibit 18).
2. Copies of laparotomy sheets - The reverse of the laparotomy sheets include visceral examinations and some of skeletal examination findings. (Exhibits 26-29)
3. Body weight data (Exhibit 24)
4. Food consumption data (Exhibit 25)
5. Visceral examination work sheet (Exhibit 31)
6. Skeletal examination data (Exhibit 30)
7. Statistical data (Exhibit 35)

The investigators audited this raw data by reconstructing the information submitted in Summary Tables. We verified total number of animals on test, independently tabulated and compared information on Summary of Uterine Implantation of all groups, verified maternal body weights, food consumption and calculated g/Kg of test substance administered. Dr. T. Collins examined selected skeletal and visceral sections.

Personnel for Study #E-89

This study was conducted by the following individuals:

1. Dr. James Vondruska - Senior Research Investigator
2. Alan L. Mitchell - Teratologist
3. Gail Kirby - Research Technician
4. Ray Schroeder - Senior Research Assistant
5. Jeanne Thompson - Research Technician

The Curriculum Vitae for Dr. James Vondruska, Alan L. Mitchell, and Gail Kirby are attached as Exhibit #16. The Curriculum Vitae for Raymond Schroeder is included with Exhibit #1.

Our review of CV's established:
Dr. James Vondruska is a licensed veterinarian and is certified by American College of Laboratory Animal Medicine. He has been employed by Searle Laboratories since March, 1973.
Dr. Vondruska said that he was responsible for submitting the final report on E-89, PT 1218S75.

Alan L. Mitchell is a graduate of Southern Illinois University and completed some graduate work at DePaul University, Chicago, Illinois. Mr. Mitchell has assisted in supervising the teratology laboratory since 1971. Regarding the conduct of E-89, Mr. Mitchell was responsible for preparing the Treatment Mixture and for supervising the maternal body weighings and food consumption.

Raymond Schroeder has a Masters degree in zoology from the University of Illinois. He worked at Searle Laboratories as a teratologist from December, 1967 until May 2, 1975. With regard to E-89, Mr. Schroeder was responsible for training Gail Kirby in teratology

and for supervising the hysterotomy examinations. A detailed account of our June 22, 1977, interview with R. Schroeder can be found in a subsequent portion of this report.

Mrs. Jeanne Thompson, Technician, has very limited duties on this study (E-89). She was responsible for taking maternal body weights and food consumption.

Gail Kirby, Research Technician, has been employed at Searle Laboratories since August, 1974. She played a major role in the conduct of E-89. In this experiment she was responsible for performing all of the visceral examinations and the skeletal exams. She received her training in teratology from Ray Schroeder.

Mrs. Kirby's educational qualifications include the following: Mrs. Kirby graduated from Elgin High School, June, 1971 and attended Loyola University for three years where she acquired 161 semester hours of credit.

During an interview with Mrs. Kirby she described her responsibilities in conducting this experiment to include the following: Mrs. Kirby told the investigators that she assisted in performing hysterotomies, weighed fetuses, sexed the fetuses, recorded gross observations, performed crown rump measurements, and recorded uterine distribution.

She stated that in E-89 she was also responsible for preparing and staining fetal skeletons and visceral sections. Mrs. Kirby initially reported that Ray Schroeder read the visceral sections on this experiment but later corrected the statement saying she examined the visceral sections.

Mrs. Kirby also told the investigators that she personally examined skeletons on this experiment and Dr. Vondruska had checked some of her observations. A detailed account of the two interviews that we held with with Gail Kirby is included in subsequent sections of this report. Curriculum vitae for J.F. Vondruska, A.L. Mitchell, and G. Kirby are attached to this report as exhibit VII. We requested the curriculum vitae for J. Thompson on numerous occasions but we were told that no formal curriculum existed for this individual.

Methods and Facilities

Interviews with Dr. James Vondruska and Alan Mitchell on 5/24/77 established the following: Dr. Vondruska stated that Vet Service Department was responsible for animal care. The diet was prepared by Alan Mitchell and he was assisted in taking body weight and food consumption data by Jeanne Thompson, Research Technician.

The investigators made an on site visit to animal facilities on 6/2/77. Dr. Vondruska identified Room 328 in J Building as the room where study E-89 was conducted.

We were shown the type of cage and feeder used. We noted that this room was equipped with temperature and lighting control. It had only one doorway for entrance and exit.

We were informed that individual female mice used in E-89 did not bear any unique identification mark after breeding. The mice were marked with tail coloring for the respective groups. Breeding cage cards and individual female cage cards are submitted as Exhibit 23. Record of daily observation would be recorded on these cards. We noted one observation on the individual cage card for animal 117 "extensive bleeding from vagina on 4/5/75". The observation is recorded in the Submission on Table No. 4 Summary Uterine Information Data Control Group.

Compound Formulations - E-89

The test substance being evaluated in this Segment II Teratology Study is L - aspartyl - L - phenylalanine methyl ester (SC18862) (aspartame) Lot 59687, Q.C. 8675. This powdered SC 18862 was administered by dietary incorporation in powdered Rat Diet from gestation day 6 through 15. The following intended dose levels were fed the test animals.

	INTENDED DAILY DOSE LEVELS	CONCENTRATION IN DIET (ACTUAL)
Low Dose	1.0 grams/Kg	.75%
Mid Dose	2.0 grams/Kg	1.50%
High Dose	4 grams/Kg	3.00%

The animals actually received approximately 40 % more than the originally intended doses.

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Searle Laboratories did not maintain batch records of the treatment/diet mixtures, nor assay for potency, homogeneity or stability. In addition we were unable to establish that the personnel kept any note books or any other written record on the method of diet preperation.

Dr. Jenkins was able to locate some uniformity of mix studies in the Mixer on a different active ingredient in Rat Chow, namely SC-10295. These results of analysis are dated March and April of 1976 and are attached as exhibit 33. Although these studies do not substantiate the uniformity of mix of SC18662 with Rat Chow, they are submitted for informational purposes.

The protocol for L-59 specifies as the mode of administration for S.C. 18662 to be admixed W/W in the diet. Dr. William Jenkins furnished the investigators with a copy of a label for the Purina Rat Chow, stating that this was the only information available as to the composition and/or specifications on the feed (Exhibit 22). Dr. Jenkins also accompanied us to the Diet Preparation Room and identified the mixer. It was a Hobart Model C-100 T with a mixing bowl of about 3 gallons capacity. Dr. Jenkins told us that there were no assays on these mixtures of S.C. 18662 with Purina Rat Chow.

Alan Mitchell told us during his interview on 5/24/77 that he was responsible for preparing the diet mix. He described his mixing procedures as follows: The diet was made up in 1000 gram batches. Approximately 500 grams was placed in the mixer bowl and then the appropriate amount of S.C. 18662 was added. Then the remaining amount of the Rat Chow was added and the contents were mixed for 10 min.

Although they did not assay the mixture of S.C. 18662 with basal diet, they did assay the test substance S.C. 18662. We obtained analytical records for their Quality Control original assay of Aspartame Lot 59687 S.C. CC075. (Exhibit #19 and Exhibit #20)

It was noted that the analyst made a decimal point error in his original work book calculations when assaying for potency. (Exhibit 19) The error was caused by using 1500 mg. instead of 150 mg. quantity in the equation and multiplying by 100 instead of 10,000. If the calculation for potency is made in accordance with the equation listed with their analytical method

Exhibit 20 and correct placement of the decimal point, the calculation indicates satisfactory potency of aspartame (S.C. 18862) for their sample weight of .1500 gms. It was explained to the investigators by Mr. Aspinall that they weighed out exactly 150 mgs. for this assay. He said that the equation for calculation of potency was not checked by a 2nd person.

Hysterotomy Data - E-89

Hysterotomies for E-89 were performed by Ray Schroeder and Gail Kirby between the periods of 3/17/75 to 4/14/75. Mrs. Kirby told the investigators during an interview on 5/24/77 that her duties for study E-89 included:

a. performing dissections
b. weighing fetuses
c. sexing fetuses
d. entering gross observations
e. did crown-rump measurements
f. recording uterine distribution.

During this time Mrs. Kirby was supervised by Ray Schroeder. Ray Schroeder was responsible for the external examination of the fetuses (see R. Schroeder interview). We authenticated the hysterotomy data by reconstructing a chart from all of the raw data. We found that this information was accurately recorded and essentially the same as in the FDA submission. We verified total number of fetuses, number of resorptions, total dead fetuses, average crown-rump measurements, and body weights (exhibits 26 through 29).

We also checked accuracy of recording the sexes by comparing the data on the laparotomy sheets against the visceral exam sheets. We found no errors in making this comparison.

Exceptions:

Female #236 with gestation day 1 of March 22, 1975 delivered 4 fetuses 4/8/75, three viable and one non viable. This appears to be a full term for the fetuses to gestation day 18. The FDA submission states that this female delivered prematurely on gestation day 18. No pups were saved for examination due to their condition. The data regarding these dead pups was not included in their calculation of means. A similar type situation was

recorded for female #308 with gestation day 1 of March 3, 1975 who delivered 10 fetuses on March 20, 1975, one cannibalized and 9 intact fetuses. This also appears to be a full term for the fetuses to gestation day 21a. No pups were saved for examination and data regarding these pups was not included in their calculations of mean values. This was probably a correct procedure because the plug of the dams was missed and hence the animals dosed on incorrect days. In Dr. Collins' opinion it would have been better if these litters had been examined and weighed and the records kept.

The uterine implantation data listed in tables in the FDA submission includes: data on number of fetuses; number of resorptions; sex distribution; mean fetal body weight; mean fetal crown-rump measurements; and number of fetal examinations. We noted the following discrepancy when we compared these tables on uterine implantation in the FDA submission with their raw data. The average female fetal crown rump measurement of animal #307 is reported on table number 4 as 2.5; it should be 2.1.

Food Consumption

Copies of the body weights and food consumption records for study L-89 are attached as Exhibit numbers 24 and 25. Our calculation of the raw data for at least one third of the food consumption quantities listed in the FDA submission indicates agreement with a statement on page 9 of the FDA submission that the pregnant animals of the low, medium and high dose groups consumed approximately 40% more than the originally intended doses of 1.0, 2.0, and 4.0 gm per kilogram.

Alan Mitchell said a 4 oz. glass jar was used as the food container. A paper under each jar was used to collect the spillage; the feed was dumped back into the feeder jar. Our calculation of original food consumption data uncovered only the following 5 discrepancies from values listed in the FDA submission.

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Animal No.	Gestation Day	Amt. of SC 18862 consumption listed in FDA submission (grams/Kg)	Amt. of SC 18862 consumption according to our calculations
405	10	4.4	4.6
232	13	1.6	2.0 * - There is an asterisk on F.C. record for day 13 - and the handwritten comment "I am not sure about spillage."
226	13	1.2	1.76
232	10	1.0	1.6 - Calculated on body wt. - day 11; day 10 body wt. was not recorded
201	6	1.3	2.25

amt ingested

Mrs. Jeanne Thompson, Research Technician, was interviewed 5/26/77. She said that she was responsible for taking body weight and food consumption data. She was supervised in these operations by Alan Mitchell. Mrs. J. Thompson said that the dosage levels of the mice were identified by marking their tails with a specific color.

Mrs. Thompson described how the animals were fed and how the weighings were done. She said that the cages were pulled out and the animals and feed container weighed on the Intec. Afterward the food was added and the container reweighed. According to her, the diet mix was stored in a labeled plastic container.

Mrs. Thompson told the investigators that where an asterisk appears on the Intec print out under food consumption, it meant that their weighing indicates spillage that is not usable for calculating food consumption. She could not recall whether or not the animals were weighed and fed at the same time each day.

Skeletal Examinations Re: Study #1-88

The results of their skeletal examinations are partly on the reverse of the laparotomy sheets. (Exhibit #26 thru #29). The research technician included a record of the date of the skeletal examination and the respective fetus number on the laparotomy sheets. However, the findings listed for the respective skeletal fetus on the back of the laparotomy sheet are for the most part incomplete because the research technician listed only the findings that she considered relatively unusual. They also have examination data in their tabular skeletal reporting format (Exhibit #30) by litter number and not by individual fetus. This tabular skeletal format is not dated. There are no initials or signatures to identify the individual who did the skeletal examinations.

We compared original examination records, the reverse of the laparotomy sheets (Exhibits #26 thru #29) and the tabular skeleton reporting format (Exhibit #30) with the report that was submitted to FDA (Exhibit #37). Dr. T. Collins made a detailed examination of skeletal specimens of 5 litters from each dose level, and authenicated the major abnormalities in other litters. Our findings include the following:

1. The original skeletal examination records essentially agree with statements in the FDA submission. The tabular skeletal reporting format (Exhibit #30) did not clearly differentiate between the total number of sternabrae centers that were absent and the total number of "small" sternebrae centers.

2. The rudimentary structures are small projections from the first lumbar vertebrae. These structures are in essence a small 14th rib. Most animals with these structures are graded twice. They are counted as having 13 pairs of ribs as well as rudimentary structures.

3. Dr. T. Collins made a detailed examination of fetal skeletal specimens from 5 litters of each dosage group. He also scanned in detail the supraoccipital bone for poor ossification in each of the skeletal fetuses of the control and high dosage group. Details regarding this examination follow in subsequent paragraphs. His examination of the supraoccipital bone revealed the following percentage differences from the FDA submission.

Supraoccipital Bone	Control Fetuses	High Dose Fetuses
Poorly Ossified		
FDA Submission	3%	6%
Examination by Dr. T. Collins	4.46%	8.47%

Dr. T. Collins also examined fetal skeletal specimens to verify their findings of major malformations: fetal skeleton #10803 with hypoplastic 4th thoracic vertebral centrum and fetal skeleton #32703, with frontal, parietal and interparietal poorly ossified, 2nd, 3rd, 4th and 5th sternebrae split, and cleft palate. He also made a rapid scan of the fetal skeleton of low dose dams #228 and #229 and medium dose dam #301 to confirm their findings.

4. Dr. Collins found some minor differences in their classification of skeletal variations. An example would be the ossification of the supraoccipital bone. A certain amount of variation normally occurs between individuals when making these types of skeletal examinations. No serious errors were found.

5. The skeletal examinations were not done blindly. The individual knew the dose levels. There is no identification on the body of each vial that each holds one skeletal specimen; the PT number 1218 and the respective fetus number are on a label on the vial cap (see exhibit 39, photo 1).

6. We made a physical inventory of the skeletal fetuses and could account for all of them with the exception of one fetus from the high level (#42210). This was reported in the FDA submission.

7. There are no dates of examination of the skeleton tables (exhibit 30). On the back of the laparotomy sheets, the major skeletal variations are listed. Most of the skeletal examinations are dated 5/19/75 and 6/4/75. It would be impossible for one individual to do a complete skeletal examination of over 500 fetuses in 2 days. It is unclear over what period of time these fetuses were read.

8. Gail Kirby, the research technician who performed the visceral and skeletal fetal examinations for study #1-89 completed about 3 years of college with job related courses that included embryology, comparative anatomy, zoology and genetics. She started employment with Searle Laboratories in August of 1974 and performed visceral and skeletal exams for study #1-89 in May and June of 1975. This was the only study where she performed the visceral exams. She stated that her-on-the job training was about 3 months. We obtained copies of two Searle Training Manuals for fetal soft tissue and skeletal examination (exhibit #32). Roger Thelen, attorney cautioned us that they couldn't determine the date when these training manuals came into existence. Therefore they couldn't be considered SOP Manuals for this study. This instruction manual does not have skeletal photos referred to in the manual.

Details for Scanning of Supraoccipital Bone in Control And High Dose Group

A selective examination was made by Dr. T. Collins for poorly ossified supraoccipital bone in all of the control (157) and high dose (119) fetal skeletons. Dr. T. Collins found ten skeletal fetuses that had a poorly ossified supraoccipital in the high dosage group; 40103, 40110, 40204, 40713, 40711, 40708, 41103, 41106, 41508 and 41603. The summary of fetal skeletal examination data in the FDA submission states that they found 7 fetuses with a poorly ossified supraoccipital bone in the high dose group. Dr. T. Collins confirmed their findings in 7 of these skeletal fetuses. He also uncovered poorly ossified supraoccipital bone in three additional skeletal fetuses in the high dose group, namely 40103, 40110 and 40713. Dr. T. Collins found seven skeletal fetuses with a poorly ossified supraoccipital in the control group; 10102, 10205, 10208, 12302, 12305, 13203, and 13208. The summary of fetal skeletal examination data in the FDA submission states that Searle found 5 skeletal fetuses from the control group with a supraoccipital bone that was poorly ossified. Dr. T. Collins confirmed their findings of 4 poorly ossified supraoccipital bone in 4 of the control skeletal fetuses. He did not agree with their finding of a poorly ossified supraoccipital bone in fetus number 10305.

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Detailed Examination of Skeletal Fetuses By Dr. T. Collins

Control Group
Number of Litters - 25
Number of Fetal Skeletons - 157
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 30
Fetus Numbers Examined:

10102	10805	11202	12302	12402
10103	10806	11203	12303	12403
10105	10808	11205	12305	12405
10106	10809	11207		12406
10108	10811			12408
10109	10813			12409
10802				12411
10803				12412
				12414

Low Dosage Group
Number of Litters - 24
Number of Fetal Skeletons - 158
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 27
Fetus Numbers Examined:

20202	21002	21802	23402	23502
20203	21003	21803	23403	23503
20205	21005	21805	23405	23505
20206	21006	21806	23407	23506
20208	21008	21808		23508
	21010	21810		23509

Medium Dosage Group
Number of Litters - 25
Number of Fetal Skeletons - 163
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 34
Fetus Numbers Examined:

30602	30702	32702	33102	33502
30603	30703	32703	33103	33503
30605	30705	32705	33105	33505
30606	30706	32706	33106	33506
30608	30708	32708	33108	33508
30609	30709		33109	33509
	30711		33111	33511
	30712		33113	

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High Dosage Group
Number of Litters - 20
Number of Fetal Skeletons - 118
Number of Litters Examined by Dr. T. Collins - 5
Number of Fetuses Examined by Dr. T. Collins - 23
Fetus Numbers Examined: 40201 41102 41201 41402 43002
40204 41103 41204 41403 43003
40207 41105 41207 41405 43005
41106 41406 43006
41408 43008
41409
41411
41412

Visceral Examination-E-89

Approximately one-third of the fetuses from each litter were fixed in Bouin's Solution for subsequent examination by the Free-Hand Sectioning Technique of Wilson. The tissue slices were examined under a dissecting microscope. All tissue slices from control and treated fetuses were then transferred to glass vials that were filled with 70% ethanol for storage. The vials are identified with the project No. PT #1218 and the respective fetus number. These tissue slices are also identified inside each of the vials with the respective fetus number.

Our physical inventory of their visceral specimens reveals that they are in conformance with the listing of the fetuses recorded on the reverse of the laporotomy sheet (Exhibit #26 thru 29). We noted that the alcohol was evaporated in the following vials and those visceral specimens might have been damaged or destroyed: 22310, 22503, 23507, 12101, 11309, 11304, 10101, 10904, 10707, 20101, 20800, 12304, 12301, 12807, 20207, 20107.

Gail Kirby who did both the visceral and skeletal examinations was aware of the dose level of the specimens that were being evaluated. There are no examination sheets that specify the abnormalities that are included in their examination of visceral sections.

During our interview with Gail Kirby she stated that a training manual had been provided her by Searle Laboratories. We subsequently received copies of training manuals from Roger Theis, Searle attorney. The firm was reluctant to provide these training manuals because they could not establish the date their manuals were initially used. Copies of their manuals for visceral and skeletal examinations were ultimately provided and are submitted Exhibit #32. In reviewing

these manuals we noted that they pertain primarily to rabbit and rat visceral exam and not to mouse visceral exam. Also, Searle Attorney, Roger Theis did not furnish copies of skeletal pictures referred to in the manual.

1. We noted only one discrepancy during our inventory. The soft tissue specimen from fetus #42269 female was found in inventory but the visceral exam records do not indicate that it had been examined. The laboratory sheet for L-69 indicates that the skeletal specimen of fetus #42210 was lost. There is a soft tissue exam listed for #42210 female with results of "O.K." for this fetus that was not in their soft tissue inventory.

   We compared the listing of the fetuses on the visceral (Exhibit #31) and laparotomy sheets (Exhibit #26-29) and noted that Searle correctly listed the same sex for the respective fetus on the visceral and laparotomy sheets. They also correctly specified the use of Bouin's fixative for the visceral specimens. We noted that the results of the visceral examinations for 9 fetuses of dam 126 and 5 fetuses of dam 226 are reported on the back of the laparotomy sheets (Exhibit #26 & 27), but these fetuses are not listed on the visceral exam sheets (Exhibit #21).

   Study R-89 was the only study where Gail Kirby performed the visceral exams.

   The visceral examination instruction manuals are not specific with regard to number of sections or thickness thru the heart. We were unable to ask Gail Kirby to examine these manuals to determine if she used them for training or reference. Mrs. Kirby was in her ninth month of pregnancy and was on maternity leave when we conducted our second and final interview by a telephone conference call to her home. Details regarding both the interviews are found in a subsequent section of this report.

Examination of Visceral Specimens by Dr. T. Collins

Dr. Collins examined a total of 31 visceral specimens. Photo #2 of Exhibit #38 illustrates some of the visceral examinations made by Dr. T. Collins.

Visceral Fetal #	Comment
32012	Dr. Collins verfied the finding of a cleft palate that was indicated in their raw data but not in their FDA submission.
40310	No Abnormalities
42207	No Abnormalities
42209	No Abnormalities
40109	Dr. Collins did not locate the section that was made for the renal pelvic area.
40301	No Abnormalities
41205	No Abnormalities
43007	They did not get enough sections.
43612	No Abnormalities
42401	No Abnormalities
42407	No Abnormalities
41906	No Abnormalities
42607	No Abnormalities
42610	Specimen was in poor condition for examination
42007	No Abnormalities
42009	No Abnormalities
40202	No Abnormalities
40206	No Abnormalities
40707	No Abnormalities
40712	No Abnormalities; but exceptionally thick sections.
41708	No Abnormalities but section of thorax was too thick, approximately 5 mm (exhibit 39, photo 2). The FDA submission stated that the slices of the thorax would be somewhat thinner than 1 mm.
20407	Dr. Collins verified the findings of a segmented uterus that was indicated in their raw data but not in their FDA submission. Dr. Collins also noted that there is a slight hydrocephalus of the ventricle and enlargement that is not in their raw data (exhibit 39, photo 3).
41101	Their raw data indicates that fetus 41101 has "a renal pelvic cavitation of the kidney not enlarged" and is an artifact and not a malformation. Examination of this fetus by Dr. Collins indicates an enlarged kidney with hydronephrosis (exhibit 39, photo 4)
43201	No Abnormalities
43204	No Abnormalities but in the opinion of Dr. Collins there were not enough sections thru the heart.

43207	No abnormalities but in the opinion of Dr. Collins there were not enough sections thru the heart.
41702	The specimen was broken up and was a problem to examine
41703	The specimen was broken up and was a problem to examine
41705	No Abnormalities
41706	No Abnormalities
41709	No Abnormalities

Dr. Collins estimated that approximately 50% of the fetuses had one or more visceral sections that were too thick (exhibit 39, photo 2). It may be significant that their findings in their total of 367 visceral sections pertained to only three fetuses (Exhibit #31). Dr. Collins noted in some cases that they missed the renal pelvic area. There is a possibility that some of the sections might have disintegrated or some of the sections might not have been placed in the vial at the time when they were originally examined by Searle Laboratoris.

Statistical Evaluation

Attached as exhibit 35 is a memo from Mr. Dennis I. Ruggles, Department of Mathematics, HFF-110 to Dr. Collins HFF-155 regarding an evaluation of the statistical methodology employed in this study (E-89). An actual statistical review was not performed. In Dr. Collins' opinion this statistical review of the FDA submission showed that the methodology employed in this study were essentially correct. The comments made by Mr. Ruggles concerning this methodology were minor (please refer to exhibit 35).

Interviews with Gail Kirby

An initial interview was held with Gail Kirby, research technician, on 5/25/77. Mrs. Kirby was reinterviewed on 6/7/77 by telephone in order to obtain additional information. We felt this was necessary because Mrs. Kirby played a major role in the conduct of E-89.

The second interview was held by a conference phone from Searle Laboratories to Mrs. Kirby at her residence.

The interviews will be reported in question and answer format to point out differences between the two interviews. Portions of this information have been reported under the respective heading. On 6/2/77 Richard Viktora, attorney told us that Gail Kirby had reconsidered her first interview and had now decided that on study E-89 she had performed the visceral examinations.

Interview with Gail Kirby 5/25/77

Q. What was your job in E-89?

A. I worked as a Research Technician in Teratology. My duties included performing hysterotomies, preparing fetuses in Bouins, preparing skeletons for staining, cutting visceral sections and recording data.

Q. Describe your hysterotomy duties.

A. These included:
1. Making dissections
2. Weighing the fetuses
3. Sexing the animal
4. Noting the gross abnormalities
5. Crown rump measurements
6. Uterine distribution of fetuses

She did the entire hysterotomy, she generally wrote her findings on the laparotomy sheet but occasionally she might have received help with the transcription.

Q. How were the Wilson sections prepared?

A. I cut the sections for someone else to look at. The sections were made as follows:

1. Six sections through head
2. 5 or 6 through thorax
3. 2 through the kidney

Q. Who evaluated the visceral sections?

A. Ray Schroeder evaluated viscerals. I may have transcribed. (Note: she subsequently stated that she made a mistake in this initial interview and that actually she did those visceral examinations).

Q. How long would it take Ray Schroeder to evaluate visceral specimens represented in 2 visceral exam sheets dated 6/17/77?

A. It would take all day. (25 litters)

Q. How did you prepare fetuses for Wilson sections?

A. Put the fetuses in bouin's for 2 or three weeks, then rinse in tap water 2 or 3 times and then cut the fetus 2 at a time on a plate. (She made a record of the fetus number).

Q. How were the fetuses sexed?

A. The sexing on the visceral was done by identifying the organ.

Q. Did you use a checklist when performing visceral exams?

A. No we did not use a form.

Q. Describe your procedure in doing visceral exam.

A. I took the fetus out of the jar which contained water. Then I sat down where I had paper on my right side. Ray Schroeder would then evaluate the visceral sections.

Q. Why don't the work sheets show more Bouins Stain?

A. I used gloves.

Q. Who did the skeletons on E-89?

A. I have done skeletons examinations, but I don't remember if I did these.

Q. Showed her the skeletal results.

A. "I did the skeletons on E-89".

Q. On your skeletal closures, what do you consider normal?

A. This criteria is given in our manuals.

4 - 75%-100% ossified
3 - 50%-75% ossified

Q. How did you assess the skull closure? Did you do a real screening job?

A. The closure was what was mostly done.

Q. Could you say you screened the frontal bone or parietal bone?

A. "I hope I did".

Q. How many autopsies could one person do in one day?

A. 30 autopsies per day. I started at 8:00 am.
(Gail said that she did not kill the animals at one time, she did the killing over an extended period of time).

Q. Who else assisted in the skeletal exam?

A. I was the only one who did skeletal exam.

Q. In doing this skeletal exam, is it fair to say that you knew what level you were looking at?

A. Yes, we knew the levels.

Q. Can you describe a 5th sternum.

A. It is always smaller, it is the size of a pin head.

Second Interview with Gail Kirby, 6/7/77
(Telephone interview)

Q. Give us your educational background.

A. I attended Loyola University until June, 1974 and accumulated some 100 hours credit at Loyola. My biology courses included comparative anatomy, embryology, microbiology, 2 inorganic chemistry courses, one organic chemistry, 2 physics, plus usual liberal arts.

Q. Did you receive a college degree?

A. No, I have not received a degree.

Q. Please tell us about your work history.

A. I started in teratology at Searle in August, 1974. My supervisor was Ray Schroeder. He taught me the basics. Ray gave me material to read and did historical control animals to show me absorption and how to make skeleton specimens. We did the visceral sections according to Wilson's book.

Q. When did you start doing skeletons.

A. Probably about 3 months after I came to Searle.

Q. Tell us how you recorded skeletal data, and the reason for having the data in two places, i.e. on back of laparotomy and in skeletal summary report by dam, e.g. 41907 5/10/75. Be noted that on back of the laparotomy sheets, there is a skeletal reading by fetus but it does not contain all of the data.

A. Each fetus was looked at individually and reported by dam number on the skeletal sheet. Once it was all tallied, anything that was unusual or outstanding was put on the back of the laparotomy sheet by fetus. The transcription was not done at time of original examination. I did not go back to the fetus to record the significant findings.

Q. How did you remember the observation?

A. I think that on that study, or the next we used a dictabelt. The fetuses were not examined twice. I transcribed and ultimately recorded the data on back of the laparotomy sheet.

Q. Regarding the visceral exams, what did you do?

A. The visceral data was also recorded in two places, i.e. on ruled sheets of paper and later put on back of laparotomy sheet. It was felt this made the data look better.

Q. On the skeletons, did you screen for supraoccipital bones. Also, what skull bones did you check for?

A. I think I have already answered that question for you. The bones of the skull are parietal, frontal, hyoid, upper jaw and lower jaw, nasal, mandible, maxilla, and the bones around the eyes. There is a listing of these on the tally sheet.

Q. Regarding your work experience, how many studies have you worked on?

A. 7 or 8 plus historical. I did visceral only on PT 1215 (E-83).

Q. How many aspartame studies have you worked on?

A. PT 1201, PT 1215.

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Note: At this point, Roger Theis, attorney, strongly objected to the line of questioning stating that this was not relevant since Dr. Collins had not found serious objections to the skeletal exam findings.

Dr. Collins stated that it was relevant because there are very few institutions where teratology training is available, consequently in house training has to be provided.

Dr. Collins asked whether or not Searle had provided a training manual giving instruction for visceral and skeletal examination. Gail Kirby told us that a manual had been available and that it contained pictures of visceral sections.

Mrs. Kirby stated that she did some controls, during which time Ray Schroeder would point out unusual findings. She stated "Ray Schroeder taught me what was a normal condition and what was not. He also taught me what to look for when making these examinations."

This concluded the telephoned interview.

Interview With Raymond Schroeder

This interview was conducted at the
on June 22, 1977. in

Q.: What was your role in study E-5? Who else was involved?

A.: I was supervisor of the group which included 2 technicians, Donna Helms and Margaret Faber Hoppenrath. I did not kill the animals but did examine the animals for external abnormalities. I read skeletons and read visceral sections after they had been cut. Donna Helms killed the animals, recorded the observations, and the food consumption, and made up the diet. Donna Helms made the crown-rump measurements by stretching the fetus out on a piece of paper towel, making two marks, and reading the distance with a caliper. Margaret Faber Hoppenrath also killed animals, made up the diet, did crown-rump measurements, and measured food consumption.

Q.: Did you do the caesareans at the same time each day?

A.: Yes, around 10 in the morning.

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Q.: What was the approximate age of the males?

A.: I have no idea of their age. The males were proven males from an in-house colony which had been used in previous studies.

Q.: Did you mix the diet?

A.: Yes, I did it initially. The aspartame was sieved because it had a tendency to ball up. The chow was not sieved. The manner of mixing was: a little chow was put into the bowl, aspartame was added and mixed for approximately 3 minutes, then the rest of the chow was added and mixed. The meal had larger particles than the aspartame and the meal was not ground. After the diet was mixed, there was no balling of the aspartame.

Q.: Were any batch records or reserve samples kept?

A.: None

Q.: How much meal was mixed up at one time?

A.: I don't know how much was mixed up at one time.

Q.: Describe the type of mixer and its location.

A.: It was a Hobart mixer, approximately 2 feet high, of approximate 10 gallon capacity. It was located on the third floor in the diet mixing room.

Q.: Was there any difference in the particle size of the aspartame and the chow?

A.: The finished mixture was homogeneous in appearance but lighter in color than regular chow. There was no balling and no rings. In my opinion the rats could not discriminate between chow and aspartame.

Q.: How were the animals placed on the racks?

A.: The animals were put on racks as they got pregnant. The racks were horizontal and the animals were put on in random fashion.

Q.: How were the animals identified in study E-5?

A.: The females were ear-punched. (He did not remember how the males were marked).

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Q.: How were the animals chosen to be mated on each dose level.

A.: The animals were placed on the experiment randomly, not by weight, and were mated 4 days per week.

Q.: Who wrote the report?

A.: I wrote the report, and also edited it.

Q.: How was it verified and collated?

A.: Dr. McConnell and I verified and collated the report.

Q.: Several errors in transposition and non-recorded data were shown to Mr. Schroeder. These included a transposition error in the recording of the unossified cervical centrum, and one unreported sternum ossification center split, 2 resorptions (in dams 57 and 58) unreported, an unreported poorly ossified ischium in dam 58, and an unreported unossified cervical centrum in the control group.

A.: I might have missed them.

Q.: How were the skeletons examined? How was the data recorded? How long was each fetus looked at?

A.: The fetus was looked at individually but the data was recorded by litter (dam). If abnormalities were found, they were identified by fetus number (e.g., fused ribs). Each fetus took approximately 5-6 minutes to observe.

Q.: Are there any sheets where the skeletal data was listed by fetus number?

A.: No

Q.: What parameters did you use for examining the visceral sections?

A.: There were no forms that were used.

Q.: Would you have seen reversed blood vessels, for example?

A.: Yes

Q.: How long did it take you to do the visceral examinations dated 2-27 and 3-5?

A.: I had many interruptions, and it obviously took longer than 2 days. I did approximately 30 per day.

Q.: Why were only 3 abnormalities reported, i.e., 2 hydrocephalus and 1 hemorrhage in the pericardial cavity?

A.: Those are the only abnormalities that were found. I think that the rat is a good rat.

Q.: Why are there differences in sex recorded on visceral sheets versus laparotomy sheets? There are approximately 20 differences.

A.: I was interrupted many times, also transposition could have take place because I was looking at 2 fetuses at the same time.

Q.: Who trained you in teratology?

A.: I trained myself by looking at many control animals plus animals from studies in 6 amino-nicotinamide, hydroxyurea, and methyl salicylate. The animals from the studies had positive tissues.

Q.: What date did you leave Searle?

A.: I left Searle on May 2, 1975.

Q.: Why did you leave Searle?

A.: I was fired by Vondruska. I didn't get along with Dr. Vondruska I left Searle in May of 1975.

Q.: What role did you play in study E-89?

A.: I did strictly external examinations, sometimes sex and weight of the fetuses, and the gross examination for external abnormalities.

Q.: Did you train Gail Kirby? If so, how?

A.: There was no formal training. I pointed out things to her and showed her representative sections. I was not there very long (approximately 4 months).

Q.: If you were in charge of teratology, would you have put Gail Kirby in charge of an entire experiment?

A.: I do not want to be pressed on answering this question. Mr. Schroeder volunteered that Gail Kirby was hired to augment the teratology group.

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Interview With Dr. James F. Vondruska

This interview was held at Searle Laboratories on July 7, 1977. Those present were Dr. Vondruska, Richard Viktora, Roger Theis, Jerry Kreshler (team leader), and Dr. Thomas Collins. Dr. Vondruska is Director of Animal Resources. At the time of the study, he was a research scientist. His immediate supervisor was Dr. Robert McConnell, Director of the Pathology-Toxicology Department.

Q.: What were the instructions given to Gail Kirby for skeletal and visceral examinations?

A.: Gail Kirby had been at Searle for several months and had been trained by Raymond Schroeder, for whom she performed the same functions. She was told to carry on. I gave her no specific instructions.

Q.: On what basis did you feel that Gail Kirby was adequately trained and had the capacity to do the skeletal and visceral sections?

A.: I relied on Schroeder's training. When he was not there, I got checked.

Q.: What percent of the 500 or more skeletons did you examine? Where are the records of your examinations?

A.: I grossly looked at 100% of the fetuses for abnormalities under a dissecting microscope. I checked a small percentage (approximately 10%) for skeletal variations. I also checked Gail Kirby's work when Schroeder wasn't there. I don't recall any records. I did not make a separate set of notes.

Q.: What percent of the 300 or so visceral sections did you examine?

A.: I did not check visceral sections. They were done by Gail Kirby Schroeder had long gone.

Q.: Did Gail Kirby use a dictaphone?

A.: She did not use a dictating machine for work performed on the bench. She made handwritten notes written on the raw data.

Q.: What was the significance of the date 6/4/75 on the front of the laparotomy sheets?

A.: This was the date on which Gail Kirby averaged the crown-rump and fetal body weights.

Q.: On what basis did you consider the renal cavitation an artifact

A.: This was probably a bad choice of terminology. I thought that Gail Kirby cut through the kidney at an incorrect angle (against the bias).

Q.: Did you examine this visceral section then?

A.: Yes

Q.: Why didn't you report the visceral malformations of segmented uterus and cleft palate in the FDA submission? (Dr. Vondreska was shown the FDA submission along with the raw data).

A.: This was probably an oversight.

Q.: What is the significance of the dates 6/14/75 and 5/19/75 on the back of the laparotomy sheets?

A.: I don't know. You will have to ask Gail Kirby.

Q.: Why did Schroeder leave?

A.: Schroeder was asked to leave. His leaving had nothing to do with technical qualifications as a researcher. He lacked supervisory skills and there were personal differences.

Interview With Margaret Faber Hoppenrath

This interview was held at her home on the evening of July 7, 1977. Present were Margaret Hoppenrath, Mr. Hoppenrath (husband), Roger Theis, Jerry Bressler (team leader), and Dr. Thomas Collins. When Mr. Bressler and Dr. Collins arrived at the Hoppenrath home at approximately 7:00 p.m., Roger Theis was already there.

Margaret Hoppenrath was mentioned by an employee as possibly having worked on study R-5. Mrs. Hoppenrath is no longer working for Searle Laboratories, but agreed to be interviewed by the FDA team. A copy of study R-5 was given to her to review on June 27, 1977.

Mrs. Hoppenrath stated that upon thinking it over, she did not think that she was involved in any of the cesareans on R-5. The

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Bart Tangonan
Tony Martinez
David Kie
Robert Spaet

The above four persons in the toxicology department were involved with assembling data for clinical chemistry and hematology determinations for April 1973 to Feb. 1974.

Joyce Schulmann - performed urinalysis and hematology determinations from April 1973 to Feb. 1974.

Philip Muellner - Technician in Path-Tox Dept. July 1970 till end of study.

Janet Praal - Technician, prepared individual work sheets for urinalysis. No longer employed by Searle.

C. The following employees were interviewed regarding clinical lab procedures, and methods for recording clinical lab. data.

1) Bart Tangonan on 6/1/77 regarding the recording of data.

2) Judith Beauchamp, on 6/2/77 regarding hematology and urinalysis.

3) Judith Schmal, on 6/2/77, 6/7/77, and 7/29/77 regarding clinical chemistry.

4) Tony Martinez, on 6/3/77 regarding urine and blood collection, and recording of data.

5) Jane Drury, on 6/7/77 regarding electrophoresis.

Accounts of these interviews are attached as exhibits #47-54.

D. Other Documents and Procedures Used to Authenticate Clinical Laboratory Data values in Submission were as follows:

1) One loose leaf volume entitled "SC-19192: 104 Week Oral Toxicity Study In The Rat. PT - 988S73 Protocols, Organ Weights, Dosage, Hematology, Urinalysis, Blood Chemistry, Protein Electrophoresis." The volume was subdivided into sections according to the above parameters. The indivi-

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13. Copy of FDA submission on study E-5

14. R & D organization chart

15. G.D. Searle Annual Report 1976

16. a) Curriculum vitae - James F. Vondruska
b) Curriculum vitae - Alan L. Mitchell
c) Curriculum vitae - Cail Kirby

17. Chain of responsibility 1975

18. Final protocol for a preclinical safety study of SC 18862, path-tox project #121857 (E-89)

19. Searle analysis of ASPARTAME C-0875, lot 59867 and copy of analyst notebook pertinent to assay, Study #E-89

20. Searle Laboratories analytical specification for Aspartame (SC 18862) method CA 02004-0574 - Study E-89

21. Charles River Breeding Lab, Wilmington, Mass.
P.O. 502726 - random bred albino mice, CD-1 strain - Study E-89

22. Label copy - Purina Rat Chow - Study E-89

23. Cage cards breed unit and individual female mouse cage card, Study E-89

24. Copy - Intec print out body weight used in E-89

25. Copy - Intec print out feed consumption data, E-89

26. Copy - laparotomy sheets Control animals - E-89

27. Copy - laparotomy sheets Low Dose animals - E-89

28. Copy - laparotomy sheet Medium Dose, E-89

29. Copy - laparotomy sheet High Dose, E-89

30. Copy - undated report - fetal skeletal examination data, E-89

31. Copy - visceral examination report, P71215, 6/5/75 - E-89

32. Copy - instruction manual fetal soft tissue and skeletal exams - E-89

33. Copy - mixer data SC 10295 in Hobart mixer - E-89

34. Copy of photo taken by Searle personnel of fetus #32703 - E-89

35. Statistical data regarding interpretation of results of Study E-89

36. Randomization procedure for Study E-89

37. Copy of FDA submission on E-89

38. Listing of data for teratology studies under FDA seal

39. Photos 1-6. Photos show identification label on cap, thick section, Hydrocephalus, Hydronephrosis, reduced ischium and missing pubic bones, Hypoplasia of the Maxilla.

40. Copy of memo refusing to allow an additional interview of Gail Kirby signed by Mr. Roger Thies.

41. Copy of authorization to examine visceral sections.

Carl E. Lorentzson
Supervisory Investigator

Johnny F. Salas

Dr. Thomas F. X. Collins